Methane Fermentation of Ammonium-Rich Organic Wastes by Zeolite

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year	2015
その他のタイトル	ゼオライトを用いた高窒素含有量有機性廃棄物のメタン発酵
学位授与大学	筑波大学 (University of Tsukuba)
学位授与年度	2014
報告番号	12102甲第7371号
URL	http://hdl.handle.net/2241/00128709

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A Dissertation Submitted to
the Graduate School of Life and Environmental Sciences,
the University of Tsukuba
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Environmental Studies
(Doctoral Program in Sustainable Environmental Studies)

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Abstract

In the past decades, with increasing in energy requirement and the issue about fossil fuels led researchers to investigate renewable energy resources. Biogas is an alternative for traditional sources of energy (petroleum, coal, natural gas, etc.) which are causing ecological-environmental problems. Today, methane fermentation is promising technique around the world, due to its useful output including renewable energy recovery and reduction pollutant emissions avoiding global warming. However, the high concentration of ammonium in manure and nitrogen-containing organic wastes frequently cause the ammonia inhibition of anaerobic digestion process, due to it is toxic to microorganisms. Therefore, the aim of this study is to mitigate ammonia inhibition for improving methane production. It is urgent task to mitigate ammonia inhibition in the anaerobic digestion process using effective techniques.

Traditional methods have been employed for alleviating ammonia inhibition, such as air stripping, adsorption, chemical precipitation, microorganism acclimation and co-digestion. Given its advantages and limitations, adding adsorption materials has been realized as a beneficial technology with expedient and economic. Comparing with other materials such as activated carbon, fly ash, and carbon nanotube, zeolite is the most promising adsorbent for ammonia removal owing to its porous structure, biochemical stability and abundance on the earth. On the other hand, zeolite seems to be a potential support material for the immobilization of microorganisms as a porous surface. These characteristics make zeolite a promising option for counteracting ammonia inhibition in the anaerobic digestion of ammonium-rich piggery wastes.

Firstly, investigate the detailed mechanisms of adsorption and efficiency of desorption on the synthesis zeolite A-3 are necessary. Ammonium adsorption on zeolite A-3 fitted with the pseudo-second-order kinetic model (R^2=0.987) and can be described by both Langmuir (R^2=0.986) and Freundlich (R^2=0.985) isotherms. The maximum adsorption capacity of ammonium nitrogen on zeolite A-3 was 78.83 mg/g at an initial NH_4^+-N concentration of 5000 mg/L. The maximum desorption efficiency (38.2%) and highest effluent NH_4^+-N concentration (76.4 mg/L) were obtained under the equilibrium state. Desorption of ammonium from saturated zeolite fits the first-order (R^2=0.982) reversible reaction kinetic.

After that, due to ammonium adsorbent of zeolite also is a potential carrier for immobilizing microorganisms, thus a zeolite-fixed bioreactor was developed by hanging zeolite A-3 fixed in a porous nylon bag (pore diameter: 3 mm) in the Duran bottle for anaerobic digestion of ammonium-rich piggery wastes. This part was carried out using two dosage loading rates 10 g/L and 30 g/L in the zeolite-fixed bioreactor and bioreactor without zeolite as control for comparing the performance. Compared with 146.4 mL/g-VS the methane yield for 33 days and startup period on the 20th day of control bioreactor, the zeolite-fixed bioreactor demonstrated good performance, with methane yield of 354.2 mL/g-VS during all 33 days of the experiment at 35°C and startup period on the 13th day. The COD removal efficiency of the zeolite-fixed bioreactor was 75.37% much higher than the control 35.10%. Using zeolite-fixed bioreactor could obviously decrease the startup period, enhanced methane yield and COD removal. In addition, the optimum zeolite loading rate 10 g/L was obtained. The ATP concentration (0.25 μmol/L) on the surface of the zeolite A-3 was much higher than that (0.026 μmol/L) in the liquid phase of the 10 g/L zeolite-fixed bioreactor. The SEM imagines confirmed that the porous surface of zeolite A-3 after anaerobic digestion was colonized by a number of methanogens. The bioreactor alleviated the ammonia inhibition during the methane fermentation of ammonium-rich piggery wastes via effective ammonium removal and immobilization of microorganisms. Direct utilization of ammonium saturated zeolite could be as fertilizer, moreover, regeneration of zeolite A-3 using Na_2SO_4 solution also obtained a $(NH_4)_2SO_4$ by-product which can be as nice nitrogenous fertilizer.

Furthermore, a new zeolite-based circulation bioreactor was developed, for eliminating ammonia inhibition and enhancing methane production in the anaerobic digestion of ammonium-rich piggery wastes. Compared with the zeolite-fixed bioreactor, it was investigated that whether the new zeolite-based circulation bioreactor could be improving the anaerobic digestion efficiency and shortening the long lag phase. As a result, the startup period of 7th day in the zeolite-based circulation bioreactor could significantly shorten 5 days compared to 12th day of zeolite-fixed bioreactor and enhanced methane production at dosage loading rates 20 g/L and 30 g/L. Compared with 5.75 L/L-bioreactor the methane production of zeolite-fixed bioreactor for 56 days, the methane production of the zeolite-based circulation bioreactors (zeolite dosage loading rate: 10 g/L, 20 g/L, 30 g/L and 50 g/L) were 5.15 L/L-bioreactor , 6.27 L/L-bioreactor, 6.69 L/L-bioreactor and 4.21 L/L-bioreactor for 56 days, respectively. According to methane production, the optimum zeolite loading rate of the zeolite-based circulation bioreactor was 30 g/L in current study. Due to characteristic of the zeolite-based circulation bioreactor, zeolite was more easily picked up as fertilizer directly or indirectly.

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Chapter 1 Introduction

1.1 Energy crisis and the importance of renewable energy resources

In a few decades, present reserve of fossil fuel energy sources will be depleted, primarily due to high demand and excessive consumption in some cases. Given that sources of fossil fuel reserves are being depleted and the greenhouse gases (GHG) emitted via their combustion is resulting in an accelerated change in global climatic conditions [1, 2], alternative sources of energy will be needed for long period. Generally, petroleum, natural gas and coal are addressed as fossil fuels [3]. The global energy consumption has increased at a geometric average of 5.6 % from 1973 onward [4]. On the basis of its growth, energy demand will rather increase promptly by one-third from 2010 to 2035, where predictably both India and China will require the highest energy supply in the world, at a rate of approximately 50 % during that period. It seems a disconcerting scene for increasing energy demand in rapidly industrialized and economically emerging countries (Fig.1.1) [5].

In addition, China is deemed to be the largest oil importer until 2020 [5]. Deserve to be mentioned, about 81.1 % of the total primary energy share was used from fossil fuel that excepts nuclear, hydro, bio fuel and other energy sources in 2010 (Fig.1.2) [6]. As shown as Fig.1.2, in 1973, percentage of the total primary energy share was 86.7 %. The share has reduced to 5.6 % in last over three decades. On the other side, 57.7 % of the energy resources in the world are accounted for transportation system [5]. This undoubted signifies a concern for the policy makers to explore alternatives that would be feasible and regenerative to achieve sustainability. Importantly, sustainability refers to maintainable provisions of energy is that fulfill the contemporary era energy demand on the premise of not affecting future generations' demands. To make it clearer we can refer to the definition of [7] which states- "development that meets the needs of the present without compromising the ability of future generations to meet their own needs". Therefore, renewable energy opened up prospects for opportune resource conservation and an eco-friendly solution directed to energy security [8]. Nevertheless, both developed and developing countries have already discerned this window of opportunity and started diverting their energy mix with renewable energy resources [9, 10].

At present, around 18 % of the global total energy consumption is developed and utilized from renewable energy resources-biomass, biofuel, hydro power, and power generation from solar energy (Fig.1.3) [3]. It is inspiring to record global investment on renewable energy resources has increased by one year increment of 17 %, thus achieving a new record of US$257 billion in 2011. Being the largest petroleum importer in the world, China is now largely investing on renewable technologies as well, with a record of US$51 billion, which is higher than what is invested in Germany, the United States, India, and Italy. In a word, renewable energy sources are becoming much popular as it has low CO_2 emission; they can provide an eco-efficient solution for developed and developing countries.

1.2 Biomass resources

As a representative material of renewable and eco-friendly, biomass can be defined as all non-fossil-based living or dead organisms and organic materials that have immanent chemical energy content. It contains that all water- and land-based organisms, vegetation and trees, or virgin biomass, and waste biomass such as municipal solid waste (MSW), bio-solids (sewage) and animal wastes (manures) and residues, forestry and agricultural residues, and certain types of industrial wastes.

Different with fossil fuel deposits, biomass is renewable in the sense that only needs a short period of time to replace what is used as an energy resource. Moreover, several environmental influences are directly associated with biomass energy production and consumption. The environmental benefit is extremely important, which contains displacing fossil fuel usage and the reduction in any disadvantageous environmental impacts that are caused by fossil fuel consumption.

Additionally, because all biomass (animal, plant and microbe), originates via CO_2 fixation by photosynthesis, thus biomass utilization is contained in the global carbon cycle of the biosphere. Consequently, the biomass conversion process is carbon balance. The global energy potential of virgin biomass is huge. The largest source of terrestrial biomass carbon is forest biomass that includes about 80 to 90% of the total biomass carbon [11] (Table 1.1). It is estimated that the world's terrestrial biomass carbon (i.e., the renewable, above–ground biomass that could be harvested and used as an energy resource) is about 100 times the world's total annual energy consumption. Hence, a significant share of our total energy need could be potentially supplied from the organic wastes produced annually. Such recycling of wastes to generate energy not only afford a source of energy, but also reduces a large number of wastes to be disposed and thereby reduces environmental problems that would have to be dealt with later.

1.3 Conversion technology of biomass

1.3.1 The classification of conversion technology

Biomass is an energy source that can either be utilized directly by combusting, or indirectly after transforming to others forms of biofuel. Conversion of biomass to biofuel can be obtained by thermal, thermochemical, and biochemical methods.

On the basic of heat as the dominant mechanism, thermal conversion processes convert biomass into another chemical form. The basic alternatives of combustion (torrefaction, pyrolysis, and gasification) are distinguished, which mainly controlled by the availability of oxygen and conversion temperature. Gasification is a clean and efficient process capable of advanced applications in developed countries and also for rural generation in developing countries [12].

In addition, biomass gasification is continually done at atmospheric pressure and causes combustion of biomass for producing a combustible gas consisting of carbon monoxide, hydrogen, and traces of methane. This gas mixture can provide fuel for a variety of vital processes, such as internal combustion engines, as well as substitute for furnace oil in direct heat applications [13]. Conversion of biomass to biofuel can also be achieved through alternative conversion of individual components of biomass

[14]. In short, thermal and thermochemical conversion pattern is economically unfeasible for its large amounts of energy consumption.

Compared with above methods, through biological process, biomass can be converted to gaseous and liquid fuels with lower energy requirements. Biochemical conversion uses the enzymes of bacteria and other microbes to degrade biomass. In most cases, microorganisms are used to carry out the conversion process: anaerobic digestion, fermentation, and composting.

1.3.2 The advantages of biological treatment

Because of the high energy recovery contacted with the process and its limited environmental influence, biological treatment technology is continually the most cost-effective [15]. The production of biogas during this process that can be used to generate electricity is the primary advantage [16, 17]. Besides that, it also has many other advantages, as follows: (1) the digested feed can be used as excellent organic fertilizer or soil improvement; (2) mitigation of waste disposal problems; (3) compare with aerobic treatment, the process does not need any oxygen; and (4) it reduces greenhouse gas emissions by displacing fossil fuels [18].

1.3.3 Biogas

As the product of anaerobic digestion process, biogas is a clean and renewable energy could commendably substitute (particularly in the village) for traditional energy sources (fossil fuels, oil, etc.) which are resulting in ecological-environmental problems and depleting at a faster rate. The definite composition of biogas is shown in Table 1.2 [19].

1.4 Environmental impact of organic wastes

Nowadays, large–scale production of pigs has been increased extremely and accompanied more and more environmental problems of piggery wastes, makes the pigs production and environmental quality are the inescapably tied together. Eutrophication of lakes, reservoirs and estuaries has raised realistic questions concerning the existence of nitrogen, phosphorus and carbon in runoff from pig production areas [20, 21, 22]. Consequently, it is extremely significant and necessary to design and construct purification system for disposing plenty of wastes.

1.5 Significance of research

The biochemical technological process of anaerobic digestion of organic substrates such as sewage and animal manures, industrial effluents and solid substrates concerns the degradation and stabilization of complex organic matter by a collection of various anaerobic microbes in the absence of oxygen [23]. The product of an energy-rich biogas with a high concentration of methane that can be used as renewable energy for replacing fossil energy sources [24]. It has been successfully performed in the treatment of the large quantities of wastes, due to its chemical oxygen demand (COD) reduction capacity and biological oxygen demand (BOD) reduction capacity from wastewater and generating renewable energy [25]. The advantages of this process is able to successfully treat wet wastes of less than 40% dry matter [26] and minimise odour with 99% of decomposing volatile compounds [27].

Although anaerobic digestion process has many advantages, however, major obstacles still remain to be resolved for the practical application of methane fermentation of organic wastes (animal manure). Generally, some factors which are significant in methane production contain the ammonium inhibition [28, 29], acidification [30], digestion conditions [31, 32, 33], and the nutritional requirements of microbes [34]. During anaerobic digestion of ammonium-rich organic wastes, a low efficient and long lag phase is often occurred, due to ammonium inhibition. However, how to effectively mitigate ammonium inhibition by excess ammonium from ammonium-rich piggery wastes is worth to do in-depth research on development bioreactor.

1.6 Methane fermentation process and mechanism

Anaerobic digestion for methane production is a biological process in which organic matter containing carbohydrates, lipids, and proteins (same with main composition of piggery wastes) is degraded to methane by the microorganism under oxygen-free conditions. The main advantage of degradation process is that a wide variety of complex organic wastes can be transformed into a single and easy available energy-rich material, meanwhile the volume of the wastes is cut down remarkably. Nowadays, anaerobic digestion has become one of the major treatment techniques for municipal sewage sludge and manure. The methane gas recovered from digestion degradation provided adequate energy to support wastewater treatment plants and farms in many cases of the world. Actually, methane produced from organic wastes has already provided whole daily energy needs for residences in some villages in China [35].

Anaerobic digestion is the consequence of a series of metabolic interactions among various groups of microorganisms. The first step is called the hydrolysis and implemented by severe anaerobes, involves the enzyme-mediated conversion of insoluble organic material and higher molecular mass compounds such as lipids, carbohydrate, polysaccharides, proteins, fats, nucleic acids, etc. into soluble organic materials, i.e. to compounds suitable for the use as source of energy and cell carbon such as monosaccharides, amino acids, fatty acids and other simple organic compounds. In the second step, acidogenesis, another group of microorganisms ferments the break-down products to acetic acid, hydrogen, carbon dioxide and other lower weight simple volatile organic acids like propionic acid and butyric acid which are in turn converted to acetic acid and hydrogen. In the third step, these acetic acid, hydrogen and carbon dioxide are converted into biogas (a mixture of methane and carbon dioxide) by the methanogenic bacteria (acetate utilizers like Methanosarcina spp. and Methanothrix spp. and hydrogen and formate utilizing species like Methanobacterium, Methanococcus, etc.) [36]. The whole conversion process of complex organic matter into methane and carbon dioxide can be separated as follow: hydrolysis, acidogenesis (acidification and acetogenesis) and methanogenesis [37], is shown in Fig.1.4.

1.7 Ammonia inhibition of anaerobic digestion

1.7.1 Overview of ammonia inhibition

Ammonia is the end-product of the biological degradation of the nitrogenous matter, such as proteins, urea and nucleic acids [38].The quantity of ammonia that will be produced from an anaerobic biodegradation of organic substrate can be estimated using the following stoichiometric relationship [25]:

$$C_aH_bO_cN_d + \frac{4a-b-2c+3d}{4}H_2O \rightarrow \frac{4a+b-2c-3d}{8}CH_4 + \frac{4a-b+2c+3d}{8}CO_2 + dNH_3$$

At lower concentration of ammonia is significant for bacterial growth, however, high concentration of ammonia may cause a poignant disturbance in the digestion degradation process i.e. cause an important decrease of microorganic activities [39, 40]. Inhibition of the anaerobic digestion process is generally demonstrated by reducing in the steady state methane production rates and increasing in the intermediate digestion products like volatile fatty acid (VFA). Toxicity is indicated by a total cessation of methanogenic activity [28, 41]. An anaerobic digester has some resemblances with the rumen of cattle. The absorption of ammonia through the rumen wall appears to avoid the occurrence of inhibitory concentrations [42]. The stability of an anaerobic process depends on the maintenance of a subtle biochemical balance between the acidogenic and methanogenic microbes. Anaerobic digestion instability can be due to the accumulation of VFA concentrations with a concurrent decrease in methane gas production.

1.7.2 Mechanism of ammonia inhibition

Many pathways for the ammonia inhibition have been mentioned, such as a change in intracellular pH of methanogens, increase of maintenance energy requirement and inhibition of a specific enzyme reaction. Ammonium ion (NH_4^+) and free ammonia nitrogen (FAN: unionized NH_3) are the two principal forms of inorganic ammonia nitrogen in aqueous anaerobic process. FAN has been suggested to be the main cause of inhibition due to its high permeability to bacterial cell membrane [43]. Knowledge of how ammonia toxicity occurs is limited and few studies with pure cultures have explained that ammonia may affect methanogenic microorganism in two ways: (1) ammonium ion may inhibit the methane generating enzymes directly and/or (2) hydrophobic ammonia molecule may permeate passively into bacterial cells, causing proton imbalance or potassium deficiency [44].

Generally, a portion of NH_3 that enters into the cells causes a pH change due to its transformation into ammonium (NH_4^+), while absorbing protons in the process. The cells must instantly consume energy to balance proton, using a potassium (K^+) pump to maintain the intracellular pH, therefore, increasing maintenance energy requirements and potentially causing inhibition of specific enzyme reactions [45]. Spread of ammonia molecules into cell wall depends upon the physiology of methanogens. Today, the research of on inhibition of anaerobic digestion process by ammonia is focused on the evolution of methanogenic populations with increasing NH_3 concentrations. From this viewpoint, Calli et al. [41] suggested that aceticlastic species might be more sensitive than hydrogenotrophic species to FAN. Karakashev et al. [46] reported that Methanosaetaceae species seems to be more sensitive among the aceticlastic to FAN accumulations than Methasorsarcinaceae, which has been found to be the dominant aceticlastic order at high NH_3 concentration (4100 mg NH_3-N/L). Similar results were obtained by Calli et al. [41] when assessing the effect of methanogenic diversity in anaerobic digesters fed with synthetic wastewater exposed to a gradual increase in NH_3 levels (ranging from 1000 to 6000 mg NH_3-N/L). Although, studies have focused on Methanosaetaceae vs. Methasorsarcinaceae dominance during high NH_3 concentrations, generally, hydrogenotrophic methanogenesis governs in the anaerobic digestion systems when operating with high NH_3 levels [46, 47]. However, the influence of NH_3 level on hydrogenotrophic methanogens has been predicted to a lower range. Wiegant and Zeeman [48] noted that Methanosarcina are large spherical cells with more volume-to-surface ratio than smaller rod-shaped Methanothrix, in short, the diffusion of FAN will be less into the Methanosarcina than Methanothrix. Hence, the removal of NH_3 would cost less energy for Methanosarcina.

1.8 Strategies for controlling the ammonia inhibition

1.8.1 Ammonia concentration

It is generally believed that ammonia concentrations below 200 mg/L are beneficial to anaerobic process since nitrogen is an essential nutrient for anaerobic microorganisms [39]. A wide range of inhibiting ammonia concentrations has been reported in the literature. McCarty [49] demonstrated that when total ammonia nitrogen (TAN) level surpasses 3000 mg NH_4^+-N/L, the anaerobic digestion processes are inhibited at any pH. In a similar study, Hobson and Shaw [50] indicated that TAN concentration of 2500 mg NH_4^+-N/L leaded to part inhibition of methane production, when a concentration up to 3300 mg NH_4^+-N/L inhibited methanogenesis absolutely. Angelidaki and Ahring [51] demonstrated that an ammonia nitrogen tolerance of up to 3000-4000 mg NH_4^+-N/L after an adapting process. These results are in accordance with the studies reported by Sung and Liu [28] and Procházka et al. [52], where they have indicated that higher TAN concentrations (>4000 mg/L) could cause apparent inhibition of methanogenesis. On the contrary, Sawayama et al. [53] and Lauterböck et al. [54] observed the inhibition when the TAN concentration exceeds 6000 mg NH_4^+-N/L. While, low ammonia nitrogen concentration (500 mg/L) can cause low methane yield, loss of biomass (as VSS) and loss of the aceticlastic methanogenic activity [52], because of negative influence of low ammonia nitrogen concentration on biomass is caused not only by low buffer capacity but also by deficiency of nitrogen as nutrient. Table 1.3 summarizes the concentrations at which ammonia are beneficial, inhibitory or toxic to the anaerobic digestion process. The significant differences of inhibiting ammonia concentrations can be attributed probably due to in nature of substrates, inoculum, environmental conditions (temperature, pH) and acclimation periods [25].

1.8.2 pH value

Instability of AD occurs at higher value of pH and it causes rapid conversion rate of ionized ammonia nitrogen into FAN. The equilibrium concentration between ammonium and FAN depends upon the process pH, as given in the Eq. (1)

$$NH_4 + OH^- \leftrightarrow NH_3 + H_2O \tag{1}$$

Further, Hansen et al. [29] reported the fraction of free ammonia relative to the total ammonia nitrogen is dependent on pH and temperature, as demonstrated in the Eq. (2)

$$NH_3(Free) = TAN \times \left(1 + \frac{10^{-pH}}{10^{-\left(0.09018 + \frac{2729.92}{T(K)}\right)}}\right)^{-1} \tag{2}$$

Here, NH_3, free ammonia nitrogen (FAN) mg/L; TAN, total ammonia nitrogen mg/L; T (K), temperature (Kelvin).

Appropriate control of pH within the growth optimum of microorganisms during anaerobic digestion process may reduce the ammonia toxicity [55]. Kayhanian [56] observed reduction in the methane yield at TAN concentration of 1000 mg/L (FAN, 60 mg/L) at pH 7.5 and 55 °C, while pH value decrease to 7.2 the FAN concentration was remained around 55 mg l^{-1}. For limiting the inhibitory effect of FAN on anaerobic digestion process, thus he suggested that the digester operated at pH 7.0. During anaerobic digestion of liquid piggery wastes (pH=8), VFAs accumulated to 316 mg/L. Adjustment pH to 7.4 resulted in reutilization of VFAs and lowered VFAs concentrations to 20 mg/L. The better performance at pH 7.4 has been attributed to the mitigation of ammonia inhibition at low pH [57]. It should also be noticed that both Methanogenic and acidogenic microorganisms have their optimal pH. Failing to maintain pH within a proper range could cause reactor failure although ammonia is at a safe level [58].

1.8.3 Temperature

Temperature is considered as a prominent factor which affects both microbial growth rates and free ammonia concentration. Generally, increasing temperature of anaerobic digestion has a positive effect on the metabolic rate of the microorganisms but also leads to a higher concentration of free ammonia concentration. Braun et al., [57] and Parkin and Miller [59] found that anaerobic fermentation of organic wastes with a high concentration of FAN was more easily inhibited and less stable at thermophilic temperatures than at mesophilic temperatures.

Gallert and Winter [60] demonstrated the 50% inhibition of anaerobic digestion of domestic bio-waste was occurred at 37 and 55 °C which corresponded to the free ammonia nitrogen concentrations of 220 and 690 mg/L, respectively. This study observed that thermophilic microbes are more resistant to higher free ammonia nitrogen concentration as compared to the mesophilic microbes. Thermophilic anaerobic digestion of slaughterhouse wastes was inhibited at 55°C, 7000 mg TAN/L or 999 mg FAN/L at pH 8.05, while anaerobic digestion at mesophilic temperature 37 °C , FAN 400 mg/L at pH value of 7.9 was disposed smoothly [61].

Even though, thermophilic anaerobic digestion could potentially for methane production, however, heating the system needs large amounts of energy, which could not be economically viable. It is also different to maintain the system sufficiency due to biological community becomes more sensitive at higher temperature.

1.8.4 Acclimation of microbes

Acclimation is another factor that can impact the degree of ammonia inhibition. A higher concentration of ammonia directly inhibits microbial activity that revealed as primary cause resulted to the digester failure, adaptation of microbes, especially the methanogens could increase the ammonia tolerance under high ammonia concentration [25, 28]. Anaerobic reactors treating low ammonia containing wastewaters may be successfully adapted to higher ammonia concentrations. Gradually, increase of ammonia concentration could enhance the adaptation of the cells. Because of the methanogens are the most sensitive among the complex microbial population linked to anaerobic digestion and the resistance to ammonia toxicity within methanogens species varies significantly. Some researches indicated the importance of bacterial adaptation to wide range of TAN/FAN levels [41, 51, 62].

In addition, the microorganisms once adapted, which can maintain viability at concentrations far overstepping the initial inhibitory concentrations [51, 63]. When unadapted methanogens failed to produce methane at 1900-2000 mg-N/L and produced methane at 11000 mg N/L after acclimation is reported by Koster and Lettinga [64]. Hashimoto [65] revealed that ammonia inhibition started at approximately 2.5 g/L and 4 g/L for unacclimated and acclimated thermophilic methanogens, respectively. After adaption, successful performance of anaerobic filters has been reached at 6 g/L and 7.8 g/L [66, 67]. Parkin and Miller [59] demonstrated that concentration as high as 8-9 g/L of total ammonia nitrogen could be endured with no significant decrease for methane production after adaptation. The experiments clearly indicated the possibility for achieving stable digestion of manure with ammonia concentration exceeding 5 g-N/L after an adaptation period. However, the methane yield was lower compared to a lower ammonia load [64, 68].

With adaptation of methanogens to ammonia could generate methane at higher than initial inhibitory concentration of ammonium, however, methane yield was low and needed long time for acclimation.

1.9 Ammonium removal methods

It is significant and necessary to remove ammonium from the ammonium-rich organic substrate for anaerobic digestion. The physical methods can be utilized by air stripping and chemical precipitation. Both have been certified to be technically viable at high ammonia concentrations and in a complex wastewater [69]. The pH value and temperature are two important factors for ammonia air stripping. But, if air stripping is carried out at high temperature, the high buffering capacity of piggery wastes could perhaps preserved pH at the required range, and the large number of alkali could be decreased. The primary restricting factor for ammonia air stripping at high temperature is the availability of cheap energy source. Besides this, with increase of temperature leading to release of more free ammonia, the toxicity to microorganisms will be caused. Although the high efficient of chemical precipitation for ammonium removal could be obtained, chemical is difficult for actual operation. In addition, a general way to ammonia inhibition relies on dilution of the manure wastes to a total solid that ranges from 0.5 to 3.0%. However, this method is unattractive economically, because of increasing waste volume that must be disposing [70].

Adding ionic exchangers or adsorbents which can remove inhibitors mitigates the ammonia inhibition [68]. Natural zeolite show high selectivity for ammonium ion and can be used as the most promising adsorbent for ammonia removal [29, 68]. Addition of antagonistic cations such as Mg^{2+} or Ca^{2+} stabilizes anaerobic degradation [71]. The positive effect of zeolite on the anaerobic process could partially be attributed to the presence of cations such as Ca^{2+} and Na^{+} that have been shown to counteract the inhibitory effect of ammonia [68].

Anaerobic ammonium oxidizing (Anammox) process has attracted concern of interests for the treatment of ammonium-rich wastewater, due to its good essences of large savings in aeration energy, organic carbon and residual sludge disposal [72, 73]. In a word, the biological processing is beneficial to environment and sustainable development. However, anammox microbes are sensitive and grow extremely slowly [74], and they are very readily inhibited by the operational conditions, such as phosphate, Chemical Oxygen Demand (COD) and nitrite etc. at high concentrations [75-77].

1.10 Objective of present research

Anaerobic digestion of piggery wastes is an attractive biological treatment technology in recent. However, the ammonia inhibition problem of piggery wastes exists all the time resulting to low efficiency and long lag phase. Therefore, the main objective of this research is to resolve the issue of ammonium inhibition from ammonium-rich piggery wastes for anaerobic digestion. The specific objectives are listed as follows. (1) Investigate the mechanisms of ammonia adsorption (kinetics and isotherms) and desorption (kinetic) on synthesis zeolite A-3. (2) Develop a zeolite-fixed bioreactor and find the optimum dosage of zeolite. (3) Develop a novel zeolite-based circulating bioreactor and evaluate its performance comparing with the zeolite-fixed bioreactor.

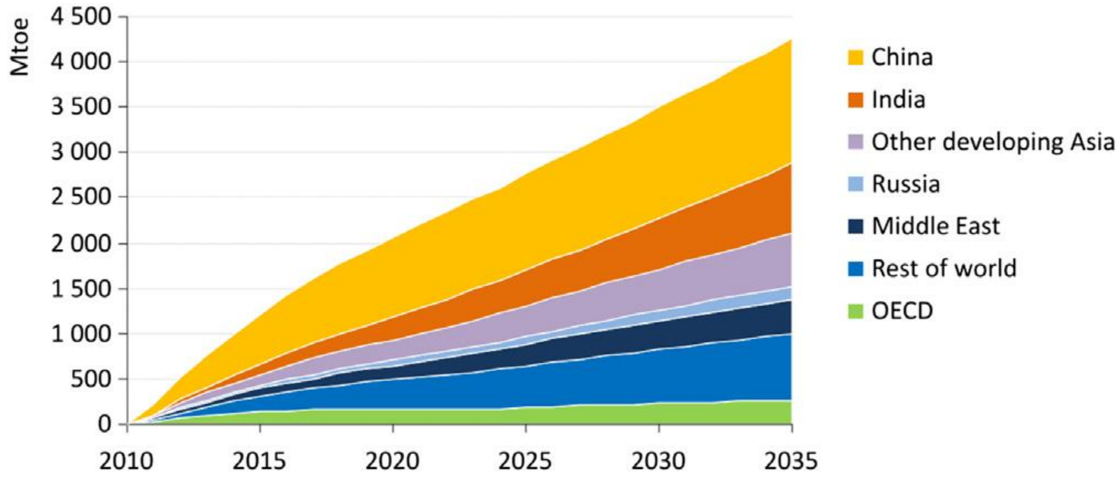


Figure 1. 1 Growth of primary energy demand [5].

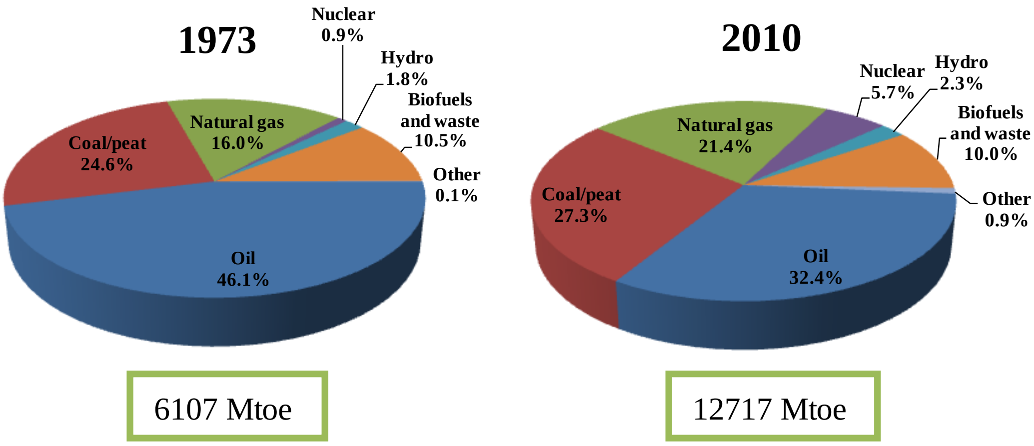


Figure 1. 2 World fuel share of total primary energy share [6].

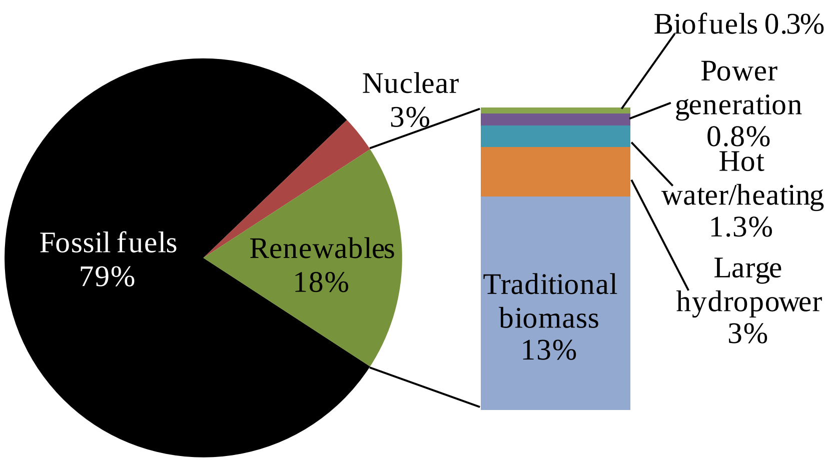


Figure 1. 3 Renewable energy share of global final energy consumption [3].

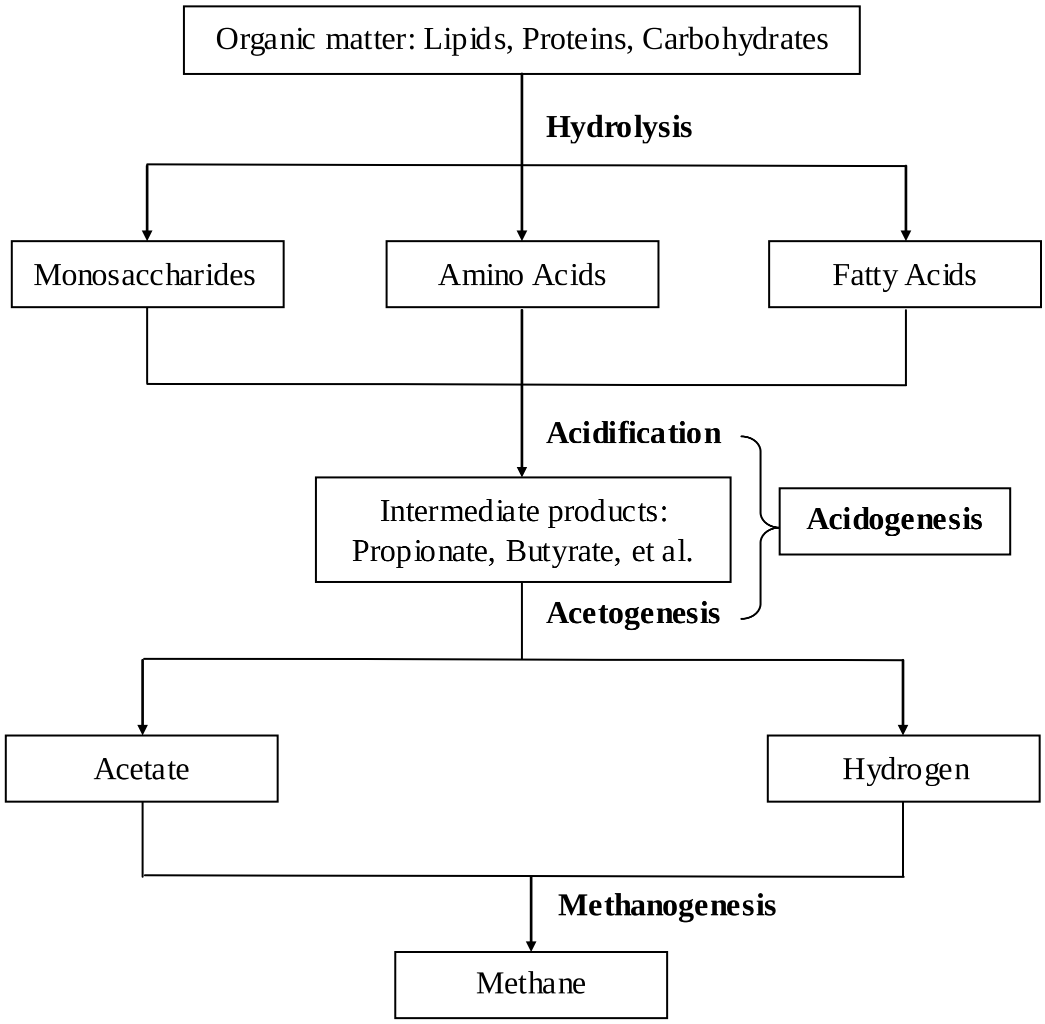


Figure 1. 4 Stages of the decomposition of piggery wastes by methane fermentation [37].

Table 1. 1 Estimated distribution of world's biomass carbon [11].

	Forests	Savanna and grasslands	Swamp and marsh	Remaining terrestrial	Marine
Area (10^6 km^2)	48.5	24.0	2.0	74.5	361
Percentage	9.5	4.7	0.4	14.6	70.8
Net C production (Gt/year)	33.26	8.51	2.70	8.40	24.62
Percentage	42.9	11.0	3.5	10.8	31.8
Standing C (Gt)	744	33.5	14.0	37.5	4.5
Percentage	89.3	4.0	1.7	4.5	0.5

Table 1. 2 Composition of biogas [19].

Constituent	Composition
Methane (CH_4)	55–75%
Carbon Dioxide (CO_2)	30–45%
Hydrogen sulphide (H_2S)	1–2%
Nitrogen (N_2)	0–1%
Hydrogen (H_2)	0–1%
Carbon monoxide (CO)	Traces
Oxygen (O_2)	Traces

Table 1. 3 Effect of ammonia levels on the anaerobic digestion process .

Effect on AD process	Ammonia (mg NH_4-N/L)	References
Beneficial	50–200	[49]
No antagonistic effect	200–1000	[50]
Inhibition (especially at higher pH values)	1500–3000	[51]
Complete inhibition or toxic at any pH	＞3000	[28,52]

Chapter 2 Adsorption and desorption studies on zeolite A–3

2.1 Introduction

Nowadays, with the rapid development of industries, large amounts of high ammonium nitrogen contained wastewater are produced. It is necessary to removal ammonium from wastewater using effective techniques for sustainable environment and health-based applications.

Conventional methods have been employed for removing ammonium such as reverse osmosis [78], break-point chlorination [79], biological nitrification [80], air stripping [81], adsorption [82], chemical precipitation [83]. Among these methods, adsorption has been utilized widely in various types of wastewater treatment for the removal of NH_4^+ [84, 85]. However, adsorption processes using organic resins as exchanger are very expensive. Therefore, cheaper materials such as zeolite and sepiolite are required [86]. The utilization of natural zeolite for removing NH_4^+ is considered to be a promising and effective treatment method due to its low cost and relative simplicity of application and operation [87, 88, 89].

Zeolites are hydrated aluminosilicates with symmetrically stacked alumina- and silica tetrahedra which result in an open and stable three-dimensional honeycomb structure [90] possessing high cation exchange capacity (CEC) and cation selectivity. In the use of zeolite, the factors which impact NH_4^+ removal performance are mainly pH, temperature, reaction time, initial concentration of NH_4^+ ions, adsorbent dosage, and other cations and anion species present in water. Each special zeolite material has its special characteristics, thus investigate the detailed mechanisms of adsorption and desorption on the synthesis zeolite A-3 is necessary.

2.2 Material and methods

2.2.1 Zeolite

The artificial zeolite A-3 used for ammonium adsorption in the experiments was provided by Wako Pure Chemical Industries, Ltd. It has the following characteristics: pore diameter (Å): 3, Particle size (mm): 2.36-4.75, absorbable molecule: H_2O, NH_3, He, unabsorbable molecule: CH_4, CO_2, C_2H_2, O_2, H_2S, C_2H_5OH, Water absorbing capacity (wt %): 20, General formula: $(0.4\ K + 0.6Na)_2O \cdot Al_2O_3 \cdot 2SiO_2$.

2.2.2 Ammonium adsorption experiment

The experiments of ammonium adsorption on zeolite A-3 were carried out in batch mode. For the ammonium nitrogen adsorption experiments and analysis, ammonium solution with a certain concentration ranging from 1000 to 5000 mg/L was prepared immediately by dissolving NH_4Cl in deionized water. Zeolite A-3 was added into 50 ml NH_4Cl solution at a loading rate of 10 g/L in a triangular flask (100 ml). Then, continuously shaking (100 rpm) of the triangular flasks were conducted in a constant temperature shaker with water bath at 35 ℃ for 24 h.

2.2.3 Ammonium desorption experiment

For nitrogen recovery in the form of ammonium sulfate ($(NH_4)_2SO_4$) which is a nice nitrogenous fertilizer, desorption of ammonium from saturated zeolite A-3 were performed in sodium sulfate solution. According to the ion equivalent exchange principle: $2NH_4^+{-}Zeolite + Na_2SO_4 = 2Na^+{-}Zeolite + (NH_4)_2SO_4$, the calculated concentration of Na_2SO_4 solution was 7.1 mol/L for the ammonium desorption from 0.5 g saturated zeolite A-3 (adsorbed NH_4^+-N: 20 mg). In the ammonium desorption experiment, 0.5 g of saturated zeolite was added into 100 ml as prepared Na_2SO_4 aqueous solution in a 200 mL triangular flask. Then, continuously shaking (100 rpm) of the triangular flasks were carried out in a shaker with water bath at 25°C for 24 h.

2.2.4 Analytical methods

The amount of ammonium nitrogen was measured by an ion meter (Ti 9001, Toyo Chemical Laboratories Co., Ltd.). The chemical of ammonium chloride used in present study was analytical grade (Wako Pure Chemical Industries, Japan), and solution was prepared in ultra-pure water (resistivity 18.2 MΩ.cm at 25°C) prepared with a water purification system (Purelite PRB-001A/002A) provided by Organo, Japan.

2.3 Results and discussion

2.3.1 Adsorption kinetic analyses on zeolite A-3

Prior to batch adsorption equilibrium studies, it is essential to confirm the equilibrium contact time required for the ammonium adsorption. Adsorption kinetic model is required for surveying the mechanism of adsorption. Several models have been utilized for the adsorption kinetic analyses. The most well-known models are Lagergren's pseudo-first-order and Ho's pseudo-second-order. In order to assess the adsorption process of ammonium on the zeolite A-3, the above two models were applied to analyze the obtained experimental data under initial ammonium concentrations of 5000 mg/L, adsorbent loading rate of 10 g/L and contact time from 0 to 24 h. The integration of the pseudo-first-order kinetic equation is expressed as [91]:

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303} t \tag{1}$$

The integration of the pseudo-second-order model can be described by the following equation:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} \tag{2}$$

where k_1 is the pseudo-first-order rate constant (min^{-1}), k_2 is the pseudo-second-order rate constant (g min^{-1}/mg), q_t is the amount of ammonium nitrogen adsorbed at time t (mg/g), q_e is the adsorption capacity at equilibrium (mg/g), and t is the contact time (min).

The regressed curves and the correlation coefficients for the pseudo-first-order and the pseudo-second-order were shown in Fig.2.1 (A, B) and Table 2.1, respectively. With regard to the pseudo-first-order model, the correlation coefficient was relatively low (R^2 = 0.905), and the experimental adsorbed masses (78.83 mg/g) was much higher than the theoretical value (34.04 mg/g) at the equilibrium time. These results indicated a bad fit between the model and the experimental data; therefore, the adsorption of ammonium on zeolite A-3 was not compliant with the pseudo-first-order reaction.

For the pseudo-second-order model, the correlation coefficient (R^2 = 0.987) was much higher than that of the pseudo-first-order model (R^2 = 0.905), and no obvious distinct occurred between the experimental (78.83 mg/g) and the theoretical adsorption capacity (77.52 mg/g) at equilibrium. The good accordance between the experimental data and the pseudo-second-order kinetic model showed that the adsorption of ammonium on the zeolite A-3 was well described by the pseudo-second-order kinetic model. As a result, this adsorption could be dominated by a chemical process, mainly ion exchange, which was in accordance with the results obtained by many other researches [92, 93].

2.3.2 Adsorption isotherms on zeolite A-3

Adsorption isotherms are essential to describe how adsorbate masses will interact with adsorbent media and are useful to optimize the use of media as adsorbents. Therefore, empirical equations such as Langmuir and Freundlich isotherm models are important for investigating the adsorption mechanism. The linearized forms of Langmuir and Freundlich isotherms were applied to analyze the adsorption process under initial ammonium concentrations ranging from 1000 to 5000 mg/L, adsorbent loading rate of 10 g/L and contact time of 24 h.

The Langmuir model assumes only one solute molecule per site, and also assumes a fixed number of sites. The linear form of the Langmuir isotherm equation can be expressed as followings [91]:

$$\frac{C_e}{q_e} = \frac{1}{bq_m} + \frac{C_e}{q_m} \tag{3}$$

Freundlich isotherm assumes that the uptakes of adsorbate occur on a heterogeneous surface by multilayer adsorption and the amount of adsorbate adsorbed increases infinitely with an increase in concentration. The linear forms of the Freundlich isotherm equation is given as:

$$\ln q_e = \ln k_f + \frac{1}{n} \ln C_e \tag{4}$$

where C_e is the liquid phase concentration of the ammonium nitrogen at equilibrium (mg/L), q_e is the amount of ammonium nitrogen adsorbed on the ceramic adsorbent at equilibrium (mg/g), q_m is the maximum adsorption capacity (mg/g), b is the Langmuir constant related to the adsorption energy (L/mg), k_f (mg$^{1\text{-}1/n}$ L$^{1/n}$ /g) is the Freundlich isotherm model constant indicating the adsorption capacity of the adsorbent, and *1/n* is an empirical parameter related to the intensity of adsorption, which varies with the heterogeneity of the material [94]. The plot of lnq_e versus lnC_e for the adsorption of ammonium nitrogen onto the zeolite A-3 was employed to generate the intercept value of k_f and the slope of *1/n*.

The fitted curves for the Langmuir and Freundlich isotherms were shown in Fig.2.2 A and B, and the isotherm parameters for the adsorption of ammonium nitrogen onto the zeolite A-3 were listed in Table 2.2. It can be seen that both Langmuir and Freundlich model were applicable for the adsorption of ammonium on the zeolite A-3, according to the high values of the regression correlation coefficients (R^2 > 0.98). The similar result was reported by Halim et al., 2010 [95], who compared the ammonia adsorption on zeolite, activated carbon and composite materials in the treatment of landfill leachate. The good compliance to Langmuir and Freundlich isotherms showed that the ammonium removal by zeolite A-3 via both the cation exchange and physical adsorption mechanism. The q_m of 84.03 mg/g calculated by the Langmuir model was higher than the measured value (78.83 mg/g). The values of the empirical parameter *1/n* lying between 0.1 < *1/n* < 1 indicated favorable adsorption for ammonium [91]. The *1/n* value (0.646) in the present study was lower than 1, which represented favorable removal conditions.

2.3.3 Ammonium desorption from saturated zeolite A-3

Sodium sulfate (Na_2SO_4) solution was used for ammonium desorption from saturated zeolite A-3, due to the advantages of nitrogen recovery in the form of ammonium sulfate ($(NH_4)_2SO_4$) which is a nice nitrogenous fertilizer and zeolite regeneration. Fig. 2.3 shows the efficiency of ammonium desorption from zeolite A-3 and effluent NH_4^+-N concentration in the bulk solution. Both the desorption efficiency of ammonium and the effluent NH_4^+-N concentration in the bulk solution increased with reaction time and gradually reached equilibrium after 20 hours. The maximum desorption efficiency (38.2%) and highest effluent NH_4^+-N concentration (76.4 mg/L) were obtained under the equilibrium state.

Desorption kinetic of NH_4^+ can be described by a first-order reversible mechanism [96], which is expressed as:

$$C_t = C_e\left(1 - e^{-(k_1+k_{-1})*t}\right) \tag{5}$$

where, C_e and C_t (mg/L) are the time-dependent concentration of the dissolved NH_4^+

at equilibrium and time t (h); k_1 and k_{-1} (h^{-1}) are the adsorption and desorption rate constants, respectively. Its logarithm form can be given as Eq. (6).

$$\ln \frac{C_e - C_t}{C_e} = -(k_1 + k_{-1}) * t \tag{6}$$

The chemical response time (τ_{resp}) for a first-order reversible reaction is:

$$\tau_{resp} = \frac{1}{(k_1 + k_{-1})} \tag{7}$$

The kinetic plot of $\ln((C_e-C_t)/C_e)$ versus t of ammonium desorption was illustrated in Fig.2.4. The high linear regression coefficient (R^2 = 0.982) indicated that desorption of ammonium from saturated zeolite A-3 well fits the first-order reversible reaction kinetic. Value for (k_1 + k_{-1}) obtained from the regression line was 0.179 h^{-1}. The reaction constant (k_1 + k_{-1}) was used in Eq. (5) to predict desorption as a function of time. The calculated τ_{resp} was 5.59 h.

2.4 Summary

Ammonium adsorption on zeolite A-3 fitted with the pseudo-second-order kinetic model and can be described by both Langmuir and Freundlich isotherms. The maximum adsorption capacity of ammonium nitrogen on zeolite A-3 was 78.83 mg/g at an initial NH_4^+-N concentration of 5000 mg/L. The maximum desorption efficiency (38.2%) and highest effluent NH_4^+-N concentration (76.4 mg/L) were obtained under the equilibrium state. Desorption of ammonium from saturated zeolite fits the first-order reversible reaction kinetic.

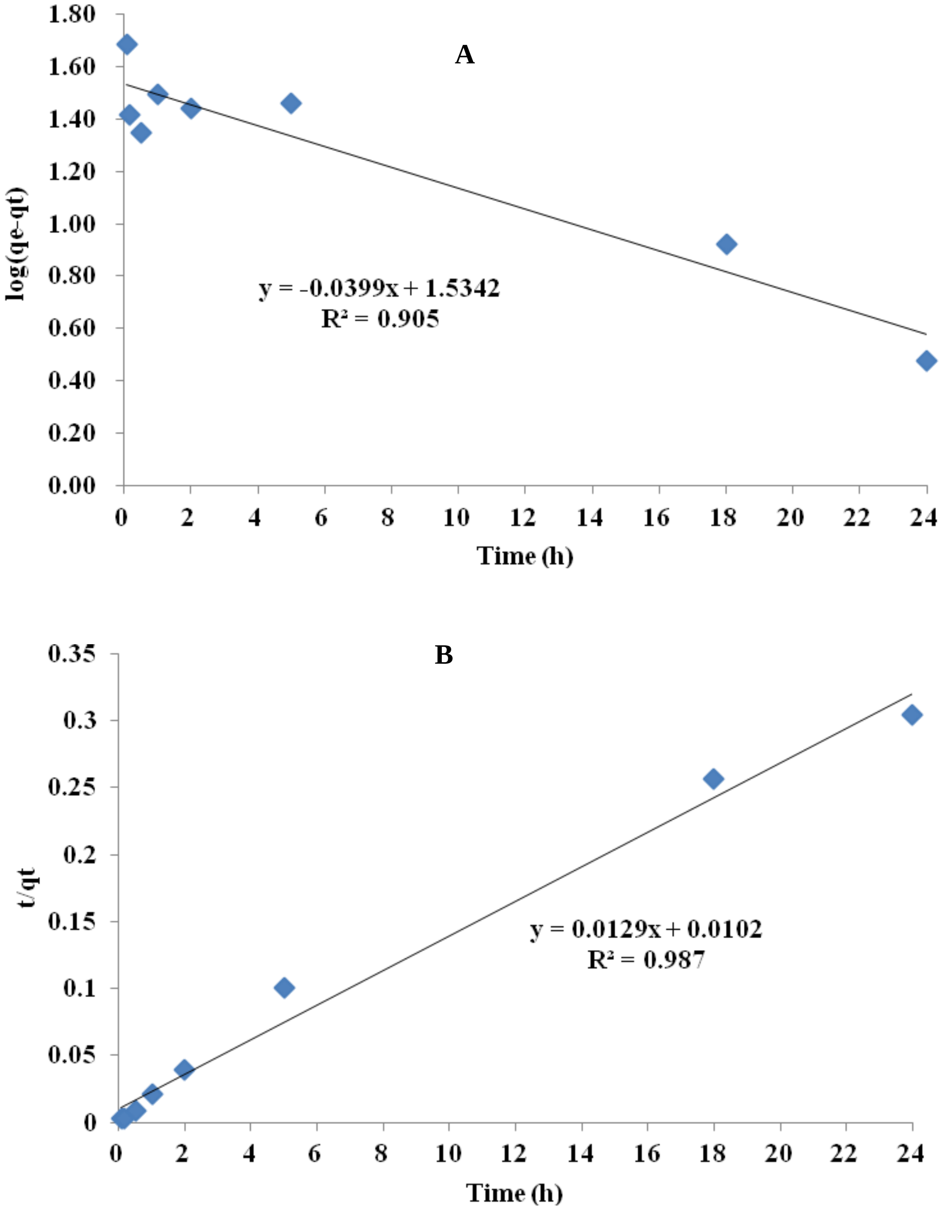


Figure 2. 1 Kinetic plots of ammonium adsorption on zeolite A-3: (A) pseudo-first-order kinetic model, (B) pseudo-second-order kinetic model.

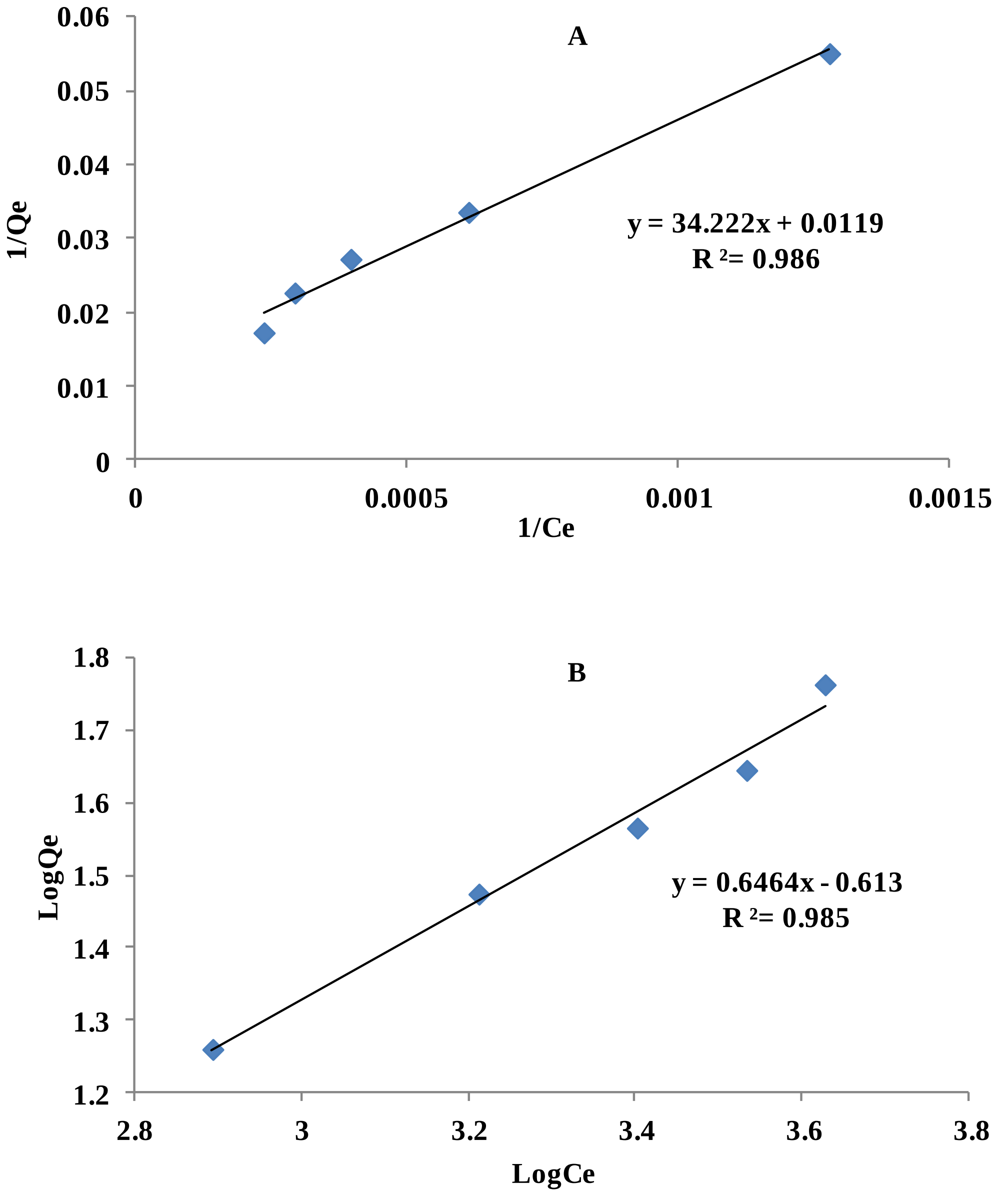


Figure 2. 2 Ammonium adsorption isotherms on zeolite A-3: (A) Langmuir isotherm model and (B) Freundlich isotherm model.

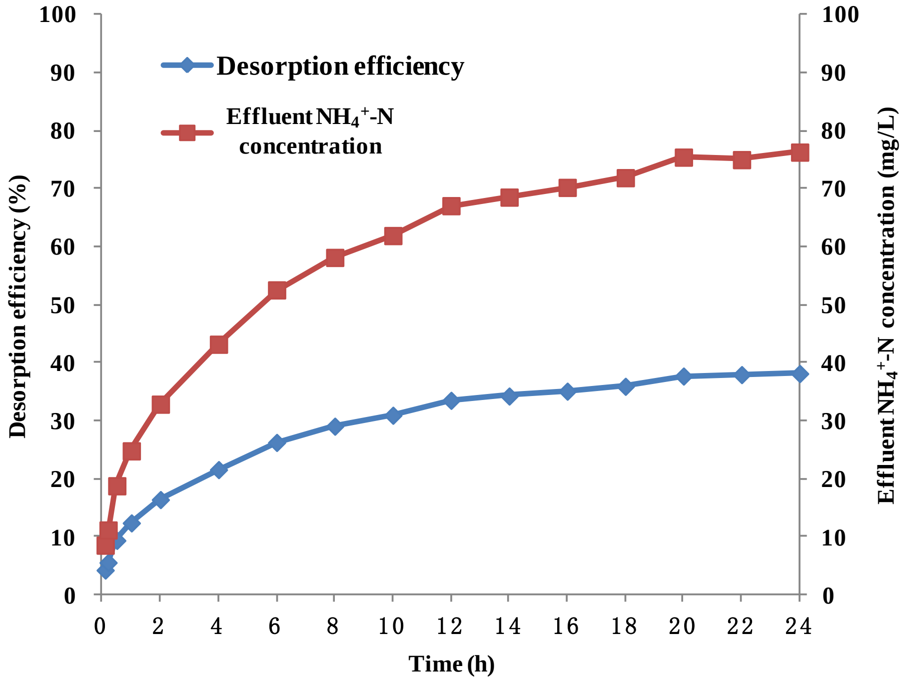


Figure 2. 3 The efficiency of ammonium desorption from zeolite A-3 and effluent NH_4^+-N concentration in the Na_2SO_4 solution.

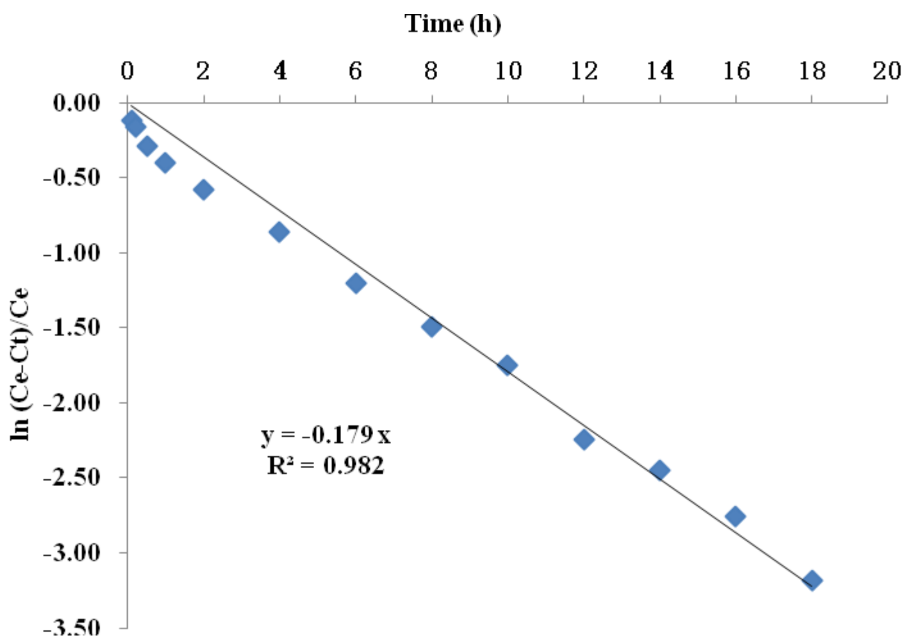


Figure 2. 4 Kinetic plots of ammonium desorption from the saturated zeolite A-3.

Table 2. 1 Pseudo-first-order model and Pseudo-second-order model constants for the ammonium adsorption on the zeolite A-3 adsorbent.

Pseudo-first-order model			Pseudo-second-order model		
K_1 (min^{-1})	q_e (mg/g)	R^2	K_2 (g/mg min^{-1})	q_e (mg/g)	R^2
0.0919	34.04	0.905	0.0163	77.5	0.987

Table 2. 2 Langmuir isotherm and Freundlich isotherm constants for the ammonium adsorption on the zeolite A-3 adsorbent.

Langmuir isotherm			Freundlich isotherm		
b (L/mg)	q_m (mg/g)	R^2	K_f ($mg^{1-1/n}$ $L^{1/n}$ /g)	1/n	R^2
0.000348	84.0	0.986	0.244	0.646	0.985

Chapter 3 Improving anaerobic methane production from ammonium–rich piggery waste in a zeolite–fixed bioreactor and evaluation of ammonium adsorbed on zeolite A–3 as fertilizer

3.1 Introduction

In the past decades, anaerobic digestion of piggery wastes has attracted considerable attention because of the bioenergy recovery in the form of methane and mitigation of environment pollution [97]. However, digestion of pure piggery wastes has been observed to be unsuccessful, due to the inhibition of ammonia produced during biodegradation of nitrogenous compounds such as proteins and amino acids [98, 99]. Although ammonia is an essential nutrient for growth of microorganisms [100], its undissociated form at high concentration has potential toxicity to methanogens [98]. Hobson and Shaw [50] reported that ammonium concentration of 2500 mg NH_4^+-N/L resulted to partial inhibition of methane production, while a complete failure of methanogenesis occurred when the concentration up to 3300 mg NH_4^+-N/L. Consequently, to improve methane production from ammonium-rich piggery wastes, it is necessary to mitigate ammonia inhibition in the anaerobic digestion process using effective techniques.

Many physicochemical and biological methods have been employed for alleviating ammonia inhibition, such as air stripping [81], adsorption [82], chemical precipitation [83], microorganisms acclimation [101] and co-digestion [102]. Among these methods, adsorption has drawn more attention because of its in-situ ammonia removal, easy operation, high safety and low cost. Comparing with activated carbon [103], fly ash [92] and activated alumina [104], zeolite is the most promising adsorbent for ammonia removal [94] owing to its porous structure, biochemical stability and abundance on the earth. On the other hand, zeolite seems to be a potential support material for the immobilization of microorganisms as a porous surface. These characteristics make zeolite a promising option for counteracting ammonia inhibition in the anaerobic digestion of ammonium-rich piggery wastes.

In recent years, effects of variety, particle size, doses and dosage procedure of zeolite addition on anaerobic digestion of piggery wastes have been investigated [105, 106, 107]. Kotsopoulos et al. [108] showed that adding natural zeolite increased methane production from piggery wastes by reducing the toxicity of ammonia and regulating the C/N (carbon/nitrogen) ratio through ammonia adsorption. Zeolite addition in anaerobic digestion of piggery wastes achieved the maximum ammonia removal at a dosage of 0.10 g-zeolite /g-VSS, regardless of particles sized [106]. Milán et al. [105] found that addition of natural zeolite (doses: 2-4 g/L) contributed to enhance the anaerobic digestion of piggery wastes by reducing the inhibitory effect of ammonia, but inhibition could not be overcome at doses higher than 6 g/L. Continuous anaerobic digestion of piggery wastes in terms of chemical oxygen demand (COD) removal efficiency and methane production was effectively promoted by addition of natural zeolite on a daily basis [109]. According to these previous studies, addition of zeolite at an appropriate dosage could effectively mitigate ammonia inhibition thereby enhance the methane production from piggery wastes. However, the enhanced methane production was only attributed to ammonium removal by zeolite, neither the immobilization of microorganism nor the fixed mode of zeolite for mitigating ammonia inhibition were investigated in all of these studies. On the other hand, ammonium desorption by using brine solution [110] is of great significance for nitrogen recovery and sustainable utilization of zeolite in the anaerobic digestion of ammonium-rich piggery wastes. Nevertheless, when using zeolite as an additive to migrate ammonia inhibition in the anaerobic digestion of piggery wastes, the ammonium desorption from zeolite had never been concerned by the previous researchers.

In this work, a zeolite-fixed bioreactor with advantages of ammonia adsorption and desorption of the adsorbed ammonium as fertilizer for future using and microorganism immobilization was developed for the anaerobic digestion of ammonium-rich piggery wastes.

3.2 Materials and methods

3.2.1 Piggery wastes and seed sludge

Ammonium-rich piggery wastes used in the experiment was stale manure that had been kept at room temperature for almost two years after it had been obtained from a pig farm located in Tokyo. The stale manure compared with fresh piggery waste has a higher concentration of ammonium which can reach levels of up to 22,310 mg/L. The piggery waste was inoculated with 25% sludge (w/w) after diluted with tap water and pH adjustment with HCl. General characteristics of the diluted substrate were: COD: 76700 mg/L, total nitrogen (TN): 9400 mg/L, total solid (TS): 35000 mg/L, volatile solid (VS): 27725 mg/L, NH_4^+-N: 3770 mg/L and pH: 7.2.

The digested sludge collected from a municipal wastewater treatment plant in Ibaraki, Japan was used as seed sludge. After it was collected, the digested sludge was storage under 4°C in a refrigerator. Before used as inoculums, 900 ml digested sludge was cultured by putting into a fermenter bottle (1000 ml). After two days, 2 g raw piggery wastes was added to this reactor every day until the methane concentration reached 80% approximately. The cultivation of methanogens was carried out at 35°C for 7 days. The characteristics of seed sludge were: COD: 6500 mg/L, TN: 5489 mg/L , TS: 9850 mg/L, VS: 7415 mg/L, NH_4^+-N: 1547 mg/L, pH: 7.1.

3.2.2 Anaerobic digestion experiment

A number of Duran bottles (300 ml, SIBATA) with silicon rubbers were used as bioreactors in this study. The methane fermentation experiments were performed in two groups of bioreactors: zeolite-fixed bioreactors and bioreactors without zeolite as the control. The zeolite-fixed bioreactor was developed by hanging zeolite A-3 fixed in a porous nylon bag (pore diameter: 3 mm) in the Duran bottle. The schematic of zeolite-fixed bioreactor was shown in Fig.3.1. In the fermentation experiments, 200 ml of diluted swine waste including 25% (w/w) digested sludge was added into each bioreactor. After that, nitrogen flush was used to keep an anaerobic condition in the bioreactors. Then, the methane fermentation of piggery wastes was carried out in a batch mode at 35°C for 33 days. The biogas was collected using 50 mL plastic syringes, and the volume was read directly using the scale on the syringe. Each group of experiments was performed in duplicate.

3.2.3 Analytical methods

The gas composition was detected by a gas chromatography (GC-8A, SHIMAZU, Japan) using a machine equipped with a thermal conductivity detector (80°C) and a Porapak-Q column (60°C). Nitrogen was used as the carrier gas. COD, TS, VS, and TN were detected according to standard methods [111], and pH was determined using a pH meter (TES 1380). The amount of ammonium nitrogen was measured by an ion meter (Ti 9001, Toyo Chemical Laboratories Co., Ltd.). The activity of microorganisms was indicated by ATP analysis using a BacTiter-Glo™ Microbial Cell Viability Assay (Promega, USA). Morphological features of microorganisms immobilized on the zeolite after anaerobic digestion was observed using a scanning electron microscope (SEM) (JSM-6330F, JEOL, Japan).

3.3 Results and discussion

3.3.1 Performance of anaerobic digestion

In a previous study [106], it was found that addition of natural zeolite (doses: 2-4 g/L) contributed to enhance the anaerobic digestion of piggery wastes with NH_4^+-N concentration of 410 mg/L by reducing the ammonium inhibitory. In this present study, the NH_4^+-N concentration of ammonium-rich piggery waste was as high as

3770 mg/L, which is approximately 9-fold of that in the previous study. Thus, to obtain an optimum addition of zeolite A-3 for methane fermentation of ammonium-rich piggery wastes, the dosages loading rates of 10 g/L and 30 g/L were used in the zeolite-fixed bioreactors. The adjusted piggery wastes with an initial ammonium nitrogen concentration of 3770 mg/L fed to each bioreactor. Ammonium inhibition has occurred above pH 7.4 within the ammonium nitrogen concentration range of 1500-3000 mg/L during the anaerobic digestion process [99].

Fig.3.2A shows that the startup period for anaerobic digestion was 13 days and 20 days in the zeolite-fixed bioreactors and the control bioreactor, respectively. Beginning from the 13^{th} day, methane production in 10 g/L and 30 g/L zeolite-fixed bioreactors increased gradually to the daily maximum of 583.5 mL/L^{-1} and 543.3mL/L on 21^{st} day, respectively. The corresponding methane concentration increased respectively from 72.5% to 87.3% and from 78.0% to 85.5% in these two bioreactors (Fig.3.2B). After that, the daily methane yield decreased gradually, whereas the methane concentration maintained at approximately 80% until the end of the digestion process. According to methane production and concentration during the first 20 days (Fig.3.2A and B), the zeolite-fixed bioreactors showed better performance than the control.

Above results indicates that the zeolite-fixed bioreactor is effective for improving methane production in anaerobic digestion of ammonium-rich piggery wastes. Because inhibitory ammonium in the anaerobic digestion of piggery wastes was partially removed by the zeolite, the NH_4^+-N levels in 10 g/L and 30 g/L zeolite-fixed bioreactors decreased respectively from 3770 to 3050 and 2958 mg/L during the first 4 day (Fig.3.2C). However, the NH_4^+-N level in the control bioreactor increased to 3896 mg/L on the 4th day. The zeolite suspended in the upper layer of the digested liquid, where ammonium could easily be removed by the adsorbent. The ammonium concentration increased during the anaerobic digestion process, because ammonia is produced by the biological degradation of the nitrogenous matter [112]. After the 4th day, the ammonium concentration in the zeolite-fixed bioreactors increased gradually. At the end of methane fermentation experiment which lasted for 33 days, the total NH_4^+-N concentration in the zeolite-fixed bioreactors (10 g/L, 30 g/L) and the control bioreactor increased to 3904, 3757 and 4940 mg/L, respectively (Fig.3.2C).

Overall, the pH value in the three bioreactors was between 7.0 and 8.1 and thus fulfilled the favorable pH level for methane fermentation (Fig.3.2D). In the zeolite-fixed bioreactors, the pH level decreased slightly (from 7.1 to 7.0) during the startup period, because of the production of volatile fatty acid (VFA). Beginning on the 15th day, the pH level gradually increased to 8.1. Then it remained at 8.1 until the end of the digestion process. The increase in pH can be explained by the increasing of ammonium concentration and the biodegradation of VFA into methane. The pH level in the control bioreactor remained constant until day 18 and then increased to 7.9 on day 33. This pH variation trend is consistent with that of methane production.

The 10 g/L and 30 g/L zeolite-fixed bioreactors showed similar trend of methane concentration and methane yield. Nevertheless, from the viewpoint of reducing mass transfer resistance and economic cost in the zeolite-fixed bioreactor, the optimum addition loading rate of zeolite was 10 g/L in this study.

3.3.2 Microorganism activity

Generally, the quantity and activity of the microorganisms in a bioreactor are two conclusive parameters [113]. ATP is an indicator of metabolically active cells and an index of microbial density, which has been shown to reflect the microorganism activity in the anaerobic digestion [114]. In this study, ATP concentration was examined on the surface of the zeolite in the zeolite-fixed bioreactor, and in the liquid from all the bioreactors at the end of the digestion experiment. The similar ATP values obtained in the liquid phase of zeolite-fixed bioreactor and control bioreactor were 0.026 and 0.023 μmol/L, respectively. However, the ATP concentration (0.25 μmol/L) on the surface of the zeolite is much higher than that in the liquid phase in the zeolite-fixed bioreactor. This indicated that the high activity levels of the immobilized microorganisms on the zeolite surface, which could be understood as pointing that the fixed zeolite is a stable and suitable carrier for microorganisms, and most of them propagated on the surface of fixed zeolite. A number of microbes assembled on the surface of fixed zeolite (Fig.3.3B) resulted to the high concentration of ATP. The distribution of microbes in the liquid phase and on the surface of the support materials were about 5% and 95% respectively [115].

On the other hand, the surface morphology of the zeolite A-3 before and after the anaerobic digestion process were observed by SEM at a magnification of 6000×. As illustrated in Fig.3.3A, the zeolite A-3 shows a porous structure and is covered with fractures. After anaerobic digestion the porous surface of zeolite was colonized subsequently by a great deal of methanogens (Fig.3.3B). This phenomenon confirmed the immobilization of microorganisms on the zeolite surface in the zeolite-fixed bioreactor.

In the anaerobic digestion of ammonium-rich piggery wastes, it has been found that free ammonia (NH_3) is the active form causing ammonia inhibition. The high concentration of free ammonia is the major causes of digester upset or failure. However, the adsorption of ammonium on zeolite surface mainly via the approach of cation exchange. Ammonium ion (NH_4^+) rather than NH_3 was adsorbed on the surface of zeolite. The toxicity of ammonia on the zeolite surface is much lower than that in the digested liquid. Therefore, the microbes tend to grow on the surface of the fixed zeolite in order to avoid the potential toxicity of free ammonia in the liquid and utilize the nitrogen source on the zeolite surface. Integrating the results of ATP analysis and SEM observation, it can be concluded that immobilization of microorganisms can be well performed using zeolite A-3 as carrier material in the zeolite-fixed bioreactor for effectively mitigating ammonia inhibition, thereby enhance the microorganism activity.

3.3.3 Effectiveness of zeolite-fixed bioreactor for the anaerobic digestion of ammonium-rich piggery wastes

As shown in Fig.3.4, the total methane yield (354.2ml/g-VS) and COD removal rate (75.37%) in zeolite-fixed bioreactor are both higher than those in the control bioreactor (146.4 ml/g-VS and 35.10%). The methane yields are lower than the theoretical value (516 ml/g-VS) for piggery wastes [116]. However, the theoretical value is based on the assumption that all of the carbon substrate transformed into methane, a fraction of the substrate is in fact used to synthesize bacterial mass [117]. In addition, the quite high initial concentrations of NH_4^+-N and COD are another factor that contributed to the lower actual methane yield for piggery wastes. Sánchez et al. [118] investigated piggery waste treatment using an upflow anaerobic sludge bed reactor (UASB) and an anaerobic fixed bed reactor (AFBR) at initial COD and NH_4^+-N concentrations less than 12600 and 650 mg/L, respectively. Their study obtained 60% COD removal in the AFBR and 40% of that in UASB, respectively. Here, at much higher initial concentrations of COD (76700 mg/L) and NH_4^+-N (3770 mg/L), the COD removal rate reached as high as 75.37% in the zeolite-fixed bioreactor. This result indicated that the zeolite-fixed bioreactor developed in this study is effective for improving the methane production from ammonium-rich piggery wastes. In further research, the practical effectiveness of developed zeolite-fixed bioreactor should be determined by carrying out the continuous anaerobic digestion of ammonium-rich piggery wastes. Due to the easy replacement and regeneration of ammonium saturated zeolite, it can be expected that the zeolite-fixed bioreactor would be stable and sustainable in continuous anaerobic digestion.

3.3.4 Evaluation of ammonium adsorbed on zeolite A-3 as fertilizer

In general, the utilization efficiency of nitrogenous fertilizer in soil in developed countries could reach 50-70% (data is from the Food and Agricultural Organization), whereas that in most of the developing countries like China is only about 30% [119]. That means most of the nitrogenous nutrient was lost when using for crop growth. Fortunately, the ammonium saturated zeolite can be directly utilized as fertilizer to effectively avoid this lost, because of its slow-release of nitrogenous nutrient into the soil [93]. Besides, the zeolite itself was considered as a soil enhancer due to its nutrient retention capacity for potassium and phosphorus [120]. In this study, the ammonium adsorption capacity of zeolite A-3 is 78.83 mg NH_4^+-N/g-zeolite, which means that approximately 7.9% of the ammonium nitrogen was adsorbed on zeolite. Direct utilization of ammonium saturated zeolite as fertilizer shows great potential to decrease annual production of nitrogen fertilizer, thereby save fertilizer cost $ 0.20 per acre-inch [121] and mitigate the environmental pollution.

On the other hand, from the viewpoint of regeneration and reuse of zeolite, desorption of ammonium from saturated zeolite is of great interest. In this present study, the regeneration of saturated zeolite A-3 was successfully achieved by desorption of ammonium into Na_2SO_4 aqueous solution. In addition, a nice nitrogenous fertilizer ($(NH_4)_2SO_4$) was obtained as a by-product during the ammonium desorption process.

3.4 Summary

A zeolite-fixed bioreactor was developed to mitigate ammonia inhibition and enhance methane production in the anaerobic digestion of ammonium-rich piggery wastes. Using zeolite-fixed bioreactor could decrease the startup period, enhanced methane yield and COD removal. Direct utilization of ammonium saturated zeolite as fertilizer could be great potential to increase the utilization efficiency of nitrogen fertilizer and decrease the environmental impact. Moreover, regeneration of zeolite A-3 using Na_2SO_4 solution also obtained a $(NH_4)_2SO_4$ by-product which is nice nitrogenous fertilizer.

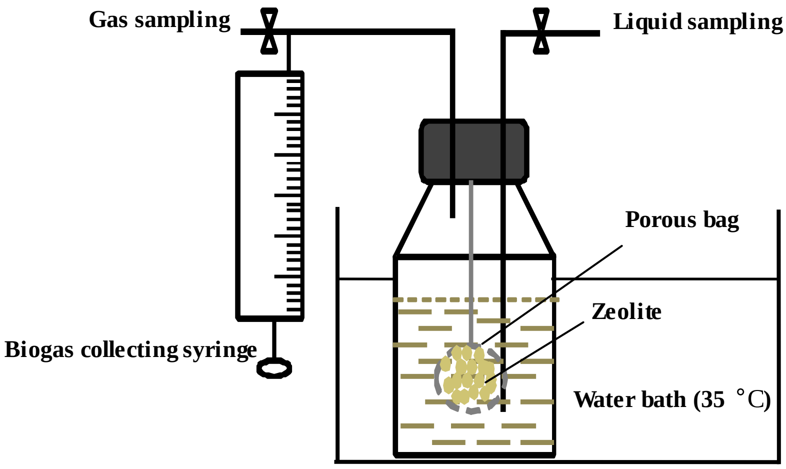


Figure 3. 1 Schematic diagram of the zeolite-fixed bioreactor.

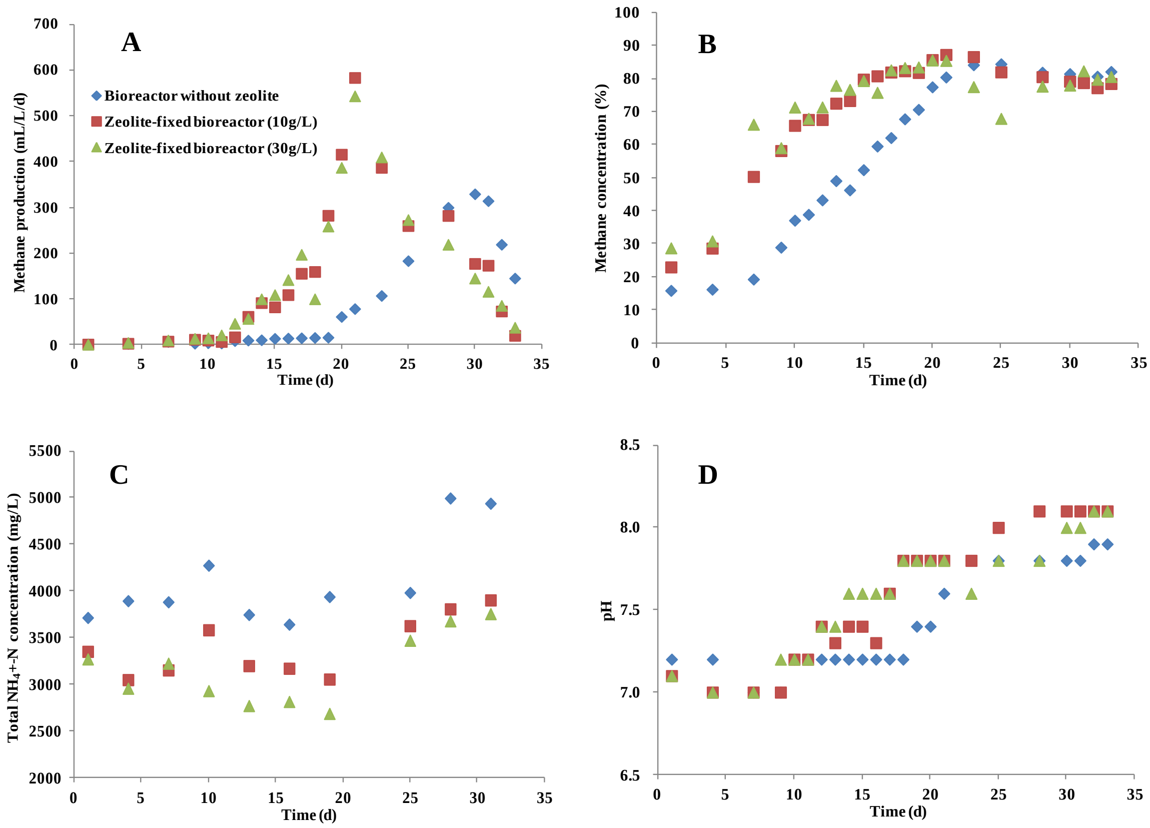
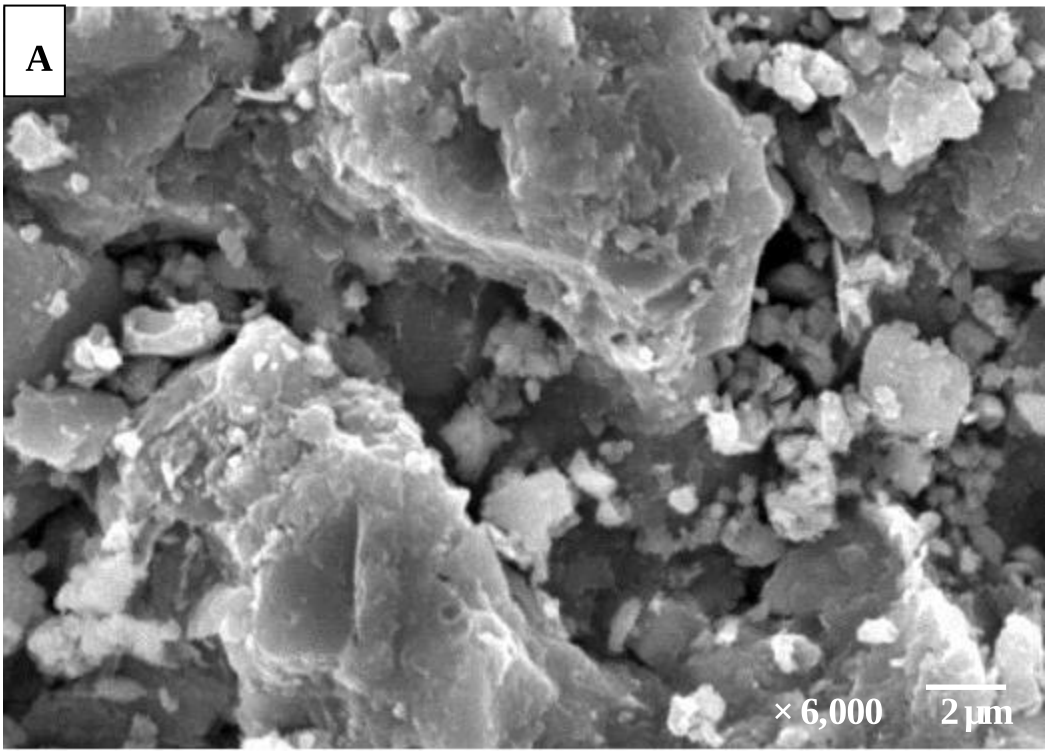


Figure 3. 2 The performance of the zeolite-fixed bioreactors and bioreactor without zeolite as control for the anaerobic digestion of piggery wastes during the experiment: (A) methane production, (B) methane concentration, (C) ammonium nitrogen concentration, (D) pH value.



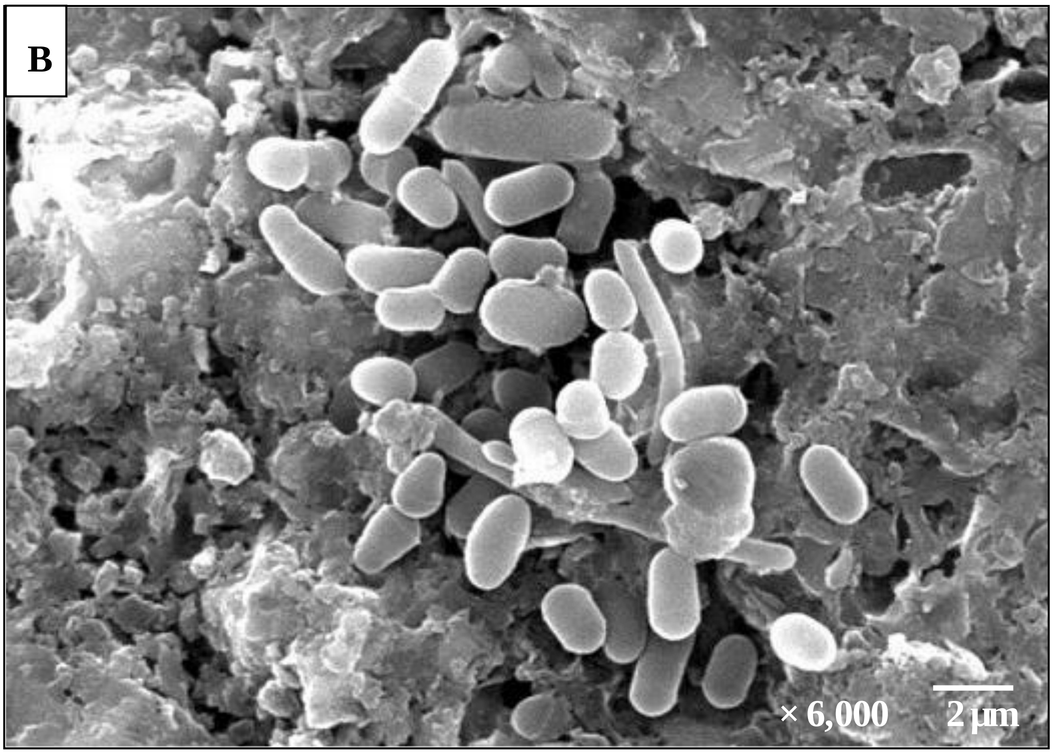


Figure 3.3 SEM images of (A) artificial zeolite A-3 and (B) microorganism immobilized in the 10g/L zeolite-fixed bioreactor.

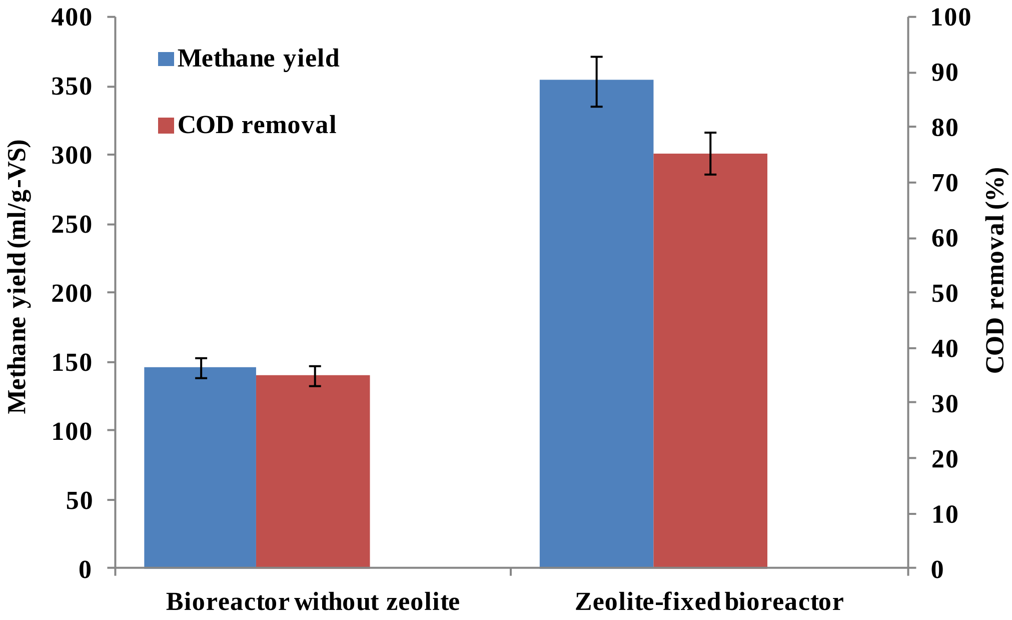


Figure 3. 4 Methane yield and COD removal in the zeolite-fixed bioreactor and bioreactor without zeolite as control.

Chapter 4 Anaerobic digestion of ammonium-rich piggery waste in a novel zeolite-based circulating bioreactor

4.1 Introduction

From the chapter 3, a zeolite-fixed bioreactor was successfully used for mitigating ammonia inhibition and enhancing methane production of ammonium-rich piggery wastes. In addition, in the anaerobic digestion system, the zeolite plays an important role such as ammonium adsorbent and immobilization microorganisms. On the other hand, chapter 3 shown that the zeolite A-3 revealed well adsorption capacity. Therefore, it is a think that simple utilize the high adsorption capacity whether could be eliminate ammonia inhibition for ammonium-rich piggery wastes. In this part, the new zeolite-based circulating bioreactor was developed because the zeolite was taken out from digestion system easily compared to zeolite-fixed bioreactor. There is no literature reported that used zeolite-based circulating bioreactor for anaerobic digestion of ammonium-rich piggery wastes.

The investigations of this part are listed as follows:

(1) Whether the new zeolite-based circulating bioreactor could improve the anaerobic digestion efficiency and shorten the long lag phase.

(2) Due to the relationship between the ammonium concentration and the zeolite dosage in the previous study [106] (was mentioned in section 3.3.1) and present research, the dosage rates 10 g/L, 20 g/L, 30 g/L and 50 g/L were used in

zeolite-based circulating bioreactor. In addition, to found the optimum dosage loading rate of zeolite A-3 in zeolite-based circulating bioreactor for facilitating comparison with the zeolite-fixed bioreactor.

4.2 Materials and Methods

4.2.1Seed sludge and piggery wastes

The digested sludge collected from a municipal wastewater treatment plant in Ibaraki, Japan was used as seed sludge. After it was collected, the digested sludge was storage under 4°C in a refrigerator. Before used as inoculums, 900 mL digested sludge was cultured by putting into a fermenter bottle (1000 mL). After two days, 2 g raw piggery wastes was added to this reactor every day until the methane concentration reached 80% approximately. The cultivation of methanogens was carried out at 35°C for 7 days. The characteristics of seed sludge were: COD: 6500 mg/L, TN: 5489 mg/L, TS: 9850 mg/L, VS: 7415 mg/L, NH_4^+-N: 1547 mg/L, pH: 7.1.

Ammonium-rich piggery wastes used in the experiment was stale manure that had been kept at room temperature for almost two years after it had been obtained from a pig farm located in Tokyo. The stale manure compared with fresh piggery waste has a higher concentration of ammonium which can reach levels of up to 22,310 mg/L. The piggery waste was inoculated with 25% sludge (w/w) after diluted with tap water and pH adjustment with 1 M HCl. General characteristics of the diluted substrate were: COD: 76210 mg/L, total nitrogen (TN):12900 mg/L, total solid (TS): 42500 mg/L, volatile solid (VS): 31500 mg/L, NH_4^+-N: 3770 mg/L and pH: 7.2.

4.2.2 Anaerobic digestion experiment

A number of Duran bottles (500 mL, SIBATA) with silicon rubbers were used as bioreactors in this study. The methane fermentation experiments were performed in two groups of bioreactors: zeolite-based circulating bioreactors and zeolite-fixed bioreactor as the control. The zeolite-based circulating bioreactor was developed by putting zeolite separated from internal anaerobic digestion system. The schematic of zeolite-based circulating bioreactor was shown in Fig.4.1. The liquid supernatant is circulated at speed of 50 mL/min. In the fermentation experiments, 200 mL of diluted piggery waste including 25% (w/w) digested sludge was added into each bioreactor. After that, nitrogen flush was used to keep an anaerobic condition in the bioreactors. Then, the methane fermentation of piggery wastes was carried out in a batch mode at 35^{o}C for 56 days. The biogas was collected using 60 mL plastic syringes, and the volume was read directly using the scale on the syringe. Each group of experiments was performed in duplicate.

4.2.3 Analytical methods

The gas composition was detected by a gas chromatography (GC-8A, SHIMAZU, Japan) using a machine equipped with a thermal conductivity detector (80 ^{o}C) and a Porapak-Q column (60 ^{o}C). Nitrogen was used as the carrier gas. COD, TS, VS, and TN were detected according to standard methods [112], and pH was determined using a pH meter (TES 1380). The amount of ammonium nitrogen was measured by an ion meter (Ti 9001, Toyo Chemical Laboratories Co., Ltd.).

Morphological features of microorganisms immobilized on the zeolite after anaerobic digestion was observed using a scanning electron microscope (SEM) (JSM-6330F, JEOL, Japan).

4.3 Results and discussion

4.3.1 The methane production of anaerobic digestion

In order to obtain an optimum dosage loading rate of zeolite A-3 for methane fermentation of ammonium-rich piggery wastes, the dosages loading rates of 10 g/L 20 g/L, 30 g/L and 50 g/L were used in the zeolite-based circulating bioreactors. The adjusted piggery wastes with an initial ammonium nitrogen concentration of 3770 mg/L fed to each bioreactor. The optimum dosage loading rate 10 g/L of zeolite-fixed bioreactor was used as control.

As show as Fig.4.2, the startup period for anaerobic digestion was 7 days and 12 days in the zeolite-based bioreactor bioreactors and the control zeolite-fixed bioreactor, respectively. Beginning from the 7th day, methane daily production in the all of zeolite-based circulating bioreactors (10 g/L 20 g/L, 30 g/L and 50 g/L) increased gradually to the daily maximum of 384.9 mL/L/d, 494.1 mL/L/d, 504.0 mL/L/d and 364.5 mL/L/d on 21th, 20th, 19th and 31th day, respectively. The accumulated methane production in the zeolite-fixed bioreactor (10 g/L) and all of zeolite-based circulating bioreactors (10 g/L 20 g/L, 30 g/L and 50 g/L) were 2.30 L, 2.06 L, 2.51 L, 2.68 L and 1.68 L, respectively.

4.3.2 The methane concentration of anaerobic digestion

The corresponding methane concentration increased respectively as follows: 53.2%-91.1%, 59.4%-89.0%, 43.9%-86.5% and 39.0-83.3%in these circulating bioreactors (Fig.4.3). After that, the daily methane yield decreased gradually, whereas the methane concentration maintained at above 60% until the end of the digestion process. In the zeolite-fixed bioreactor, the maximum daily production was 368.5 mL/L/d on 32th, and the methane concentration was increased from 61.3% to 93.3% before 32th. In the initial stage, the methane concentration of the zeolite-fixed bioreactor is slightly higher than circulating bioreactors, it is inferred that air enters into the internal of system while circulating the liquid supernatant. However, there is no influence on daily methane production later. According to methane production (Fig.4.2) and methane concentration (Fig.4.3) during the first 20 days, the zeolite-based circulating bioreactors (10 g/L, 20 g/L and 30 g/L) showed better performance than the control. And the zeolite-based circulating bioreactors observably shortened lag phase compared to control.

4.3.3 The ammonium nitrogen concentration variation of anaerobic digestion

Above results indicates that the zeolite-based circulating bioreactor is effective for improving methane production in anaerobic digestion of ammonium-rich piggery wastes. Because inhibitory ammonium in the anaerobic digestion of piggery wastes was mostly removed by the zeolite. The NH_4^+-N concentrations have similar trend in 10 g/L, 20 g/L and 30 g/L zeolite-based circulating bioreactors which the lowest decreased respectively from 3770 mg/L to 1638 mg/L, 1515 mg/L and 1624 mg/L on the 28th (Fig.4.4). However, the NH_4^+-N level in the control bioreactor decreased to 2255 mg/L on the 15th day. The zeolite was moved out from internal system, could be get effect instantly for relieving ammonia inhibition.

4.3.3 The pH variation of anaerobic digestion

In general, the value in the five bioreactors was between 7.0 and 8.3 and thus fulfilled the favorable pH level for methane fermentation (Fig.4.5). Beginning on the 9th day, the pH level gradually increased to 8.0-8.2 in the zeolite-based circulating bioreactor. The increase in pH can be explained by the increasing of ammonium concentration and the biodegradation of VFA into methane. The pH level in the control bioreactor remained slowly increased then increased to 8.2 on day 56. The present pH variation trend is consistent with that of methane production.

The methane production of the zeolite-based circulating bioreactors (10 g/L, 20 g/L, 30 g/L and 50 g/L) was calculated as 5.15 L/L, 6.27 L/L, 6.69 L/L and 4.21 L/L, respectively. The 20 g/L and 30 g/L zeolite-based circulating bioreactors showed higher than control, which value was 5.75 L/L far higher than 50 g/L of zeolite-based circulating bioreactor. This was indicated that increasing zeolite additive the methane production not follow increasing always, because of the volume of filling zeolite carrier in zeolite-based circulating bioreactor. 50 g/L of zeolite-based circulating bioreactor had lowest methane production possibly due to increase mass transfer resistance. Therefore, in allusion to the zeolite-fixed bioreactor, the optimum addition loading rate of zeolite was 30 g/L in current study.

4.3.4 SEM images

Fig.4.6 shown that the surface morphology of the zeolite A-3 before and after the anaerobic digestion process in zeolite-based circulating bioreactor were observed by SEM at a magnification of 6000×. As Fig.4.6A illustrated that the zeolite A-3 demonstrates a porous structure and is covered with fractures. After anaerobic digestion, the microorganisms were not attaching themselves on the porous structure of zeolite (Fig.4.6 B). Whether the microorganisms could be stay for a time on the surface of zeolite is considered. If likes that will be result to reduce methane production due to decrease the amount of microorganisms.

4.3.5 Comparison of the zeolite-fixed bioreactor and the zeolite-based circulating bioreactor

The comparison between the zeolite-fixed bioreactor and the zeolite-based circulating bioreactor were shown in Table 4.1. The startup period of the zeolite-based circulating bioreactor (7^{th} day) was much earlier than the zeolite-fixed bioreactor (12^{th} day). Moreover, the accumulated methane production was higher in zeolite-based circulating bioreactor. However, from the viewpoint of cost, the zeolite-fixed bioreactor is economic. Because of the optimum zeolite dosage was 10 g/L, less than the zeolite-based bioreactor (30 g/L). Due to the microorganism immobilized on the surface of zeolite in the fixed reactor, the methane concentration was also a little higher than the zeolite-based circulating bioreactor. Therefore, combined the advantages of the two bioreactors, the zeolite-fixed circulating bioreactor was suggested for future use.

4.4 Summary

A new zeolite-based circulating bioreactor was developed for eliminating ammonia inhibition and enhancing methane production in the anaerobic digestion of ammonium-rich piggery wastes. The zeolite-based circulating bioreactor could shorten the startup period compared with zeolite-fixed bioreactor and enhanced methane production. The optimum zeolite loading rate of the zeolite-fixed bioreactor was 30 g/L in current study. In addition, zeolite was more easily picked up from zeolite-based circulating bioreactor as fertilizer directly or indirectly.

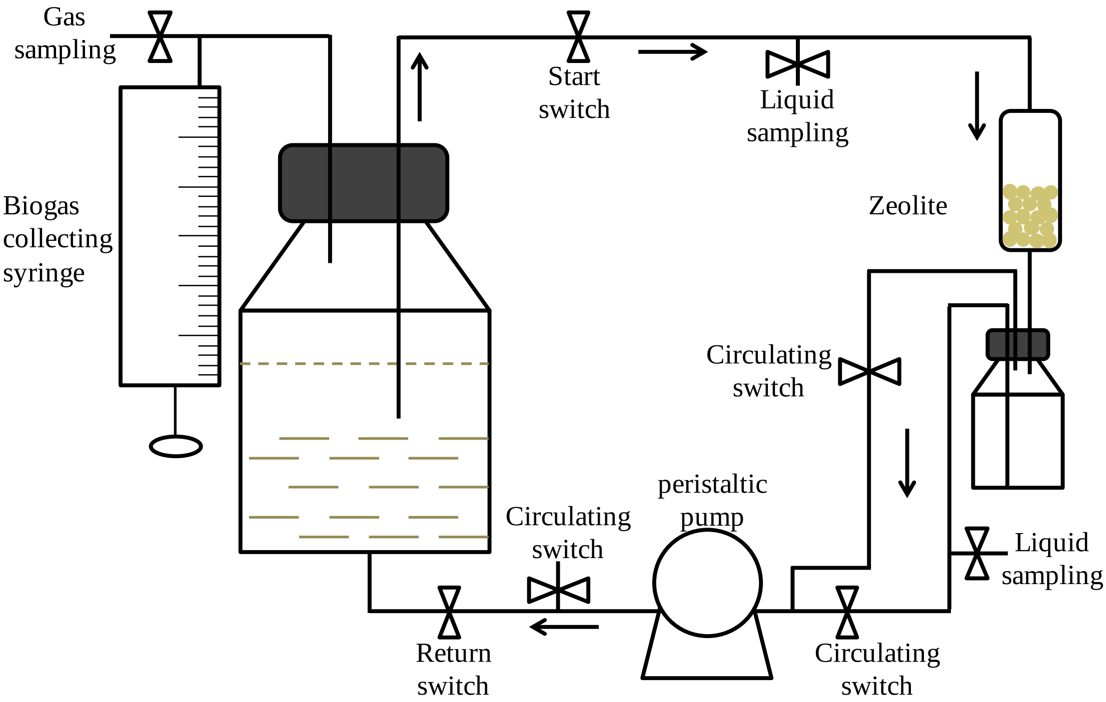


Figure 4. 1 Schematic of zeolite-based circulating bioreactor.

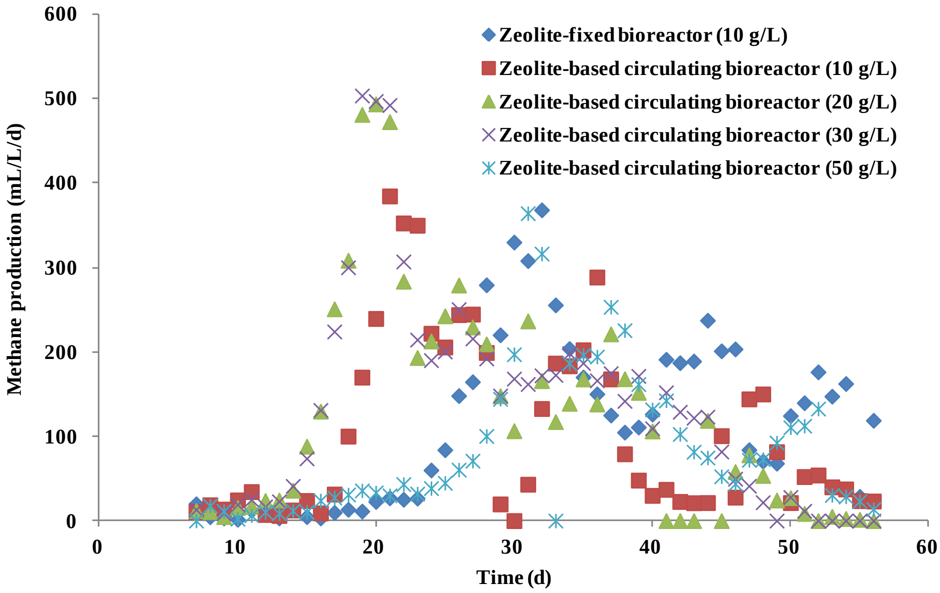


Figure 4. 2 The methane production of the zeolite-based bioreactors and zeolite-fixed bioreactor as control for the anaerobic digestion of piggery wastes during the experiment.

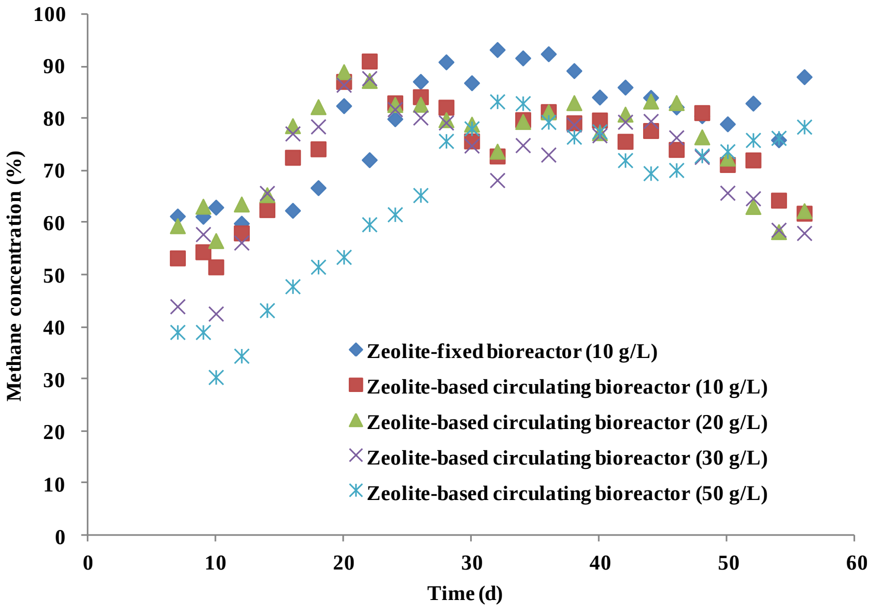


Figure 4. 3 The methane concentration of the zeolite-based bioreactors and zeolite-fixed bioreactors as control for the anaerobic digestion of piggery wastes during the experiment.

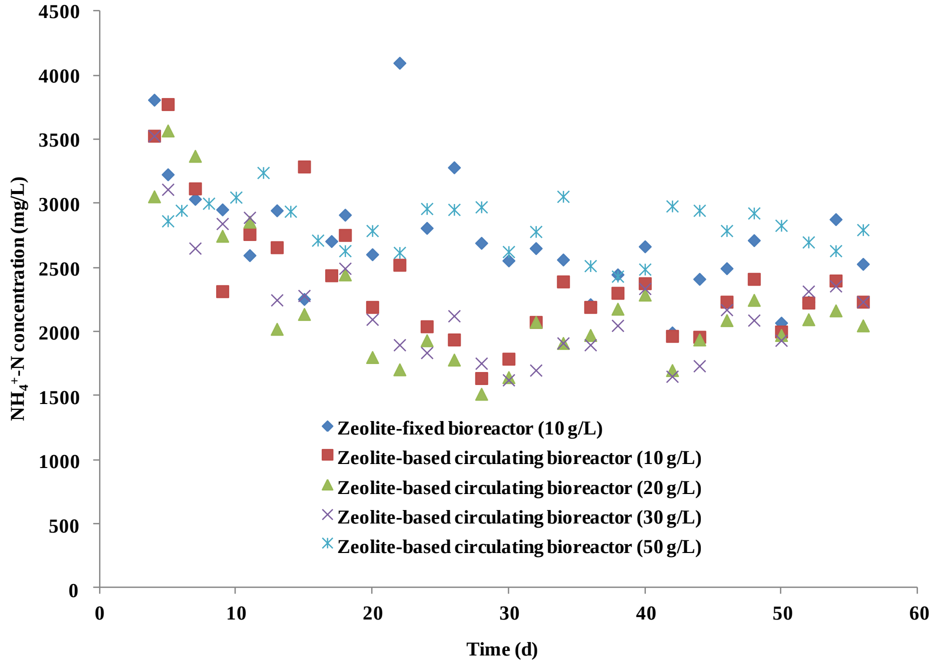


Figure 4. 4 The ammonium nitrogen concentration variation of the zeolite-based bioreactors and zeolite-fixed bioreactor as control for the anaerobic digestion of piggery wastes during the experiment.

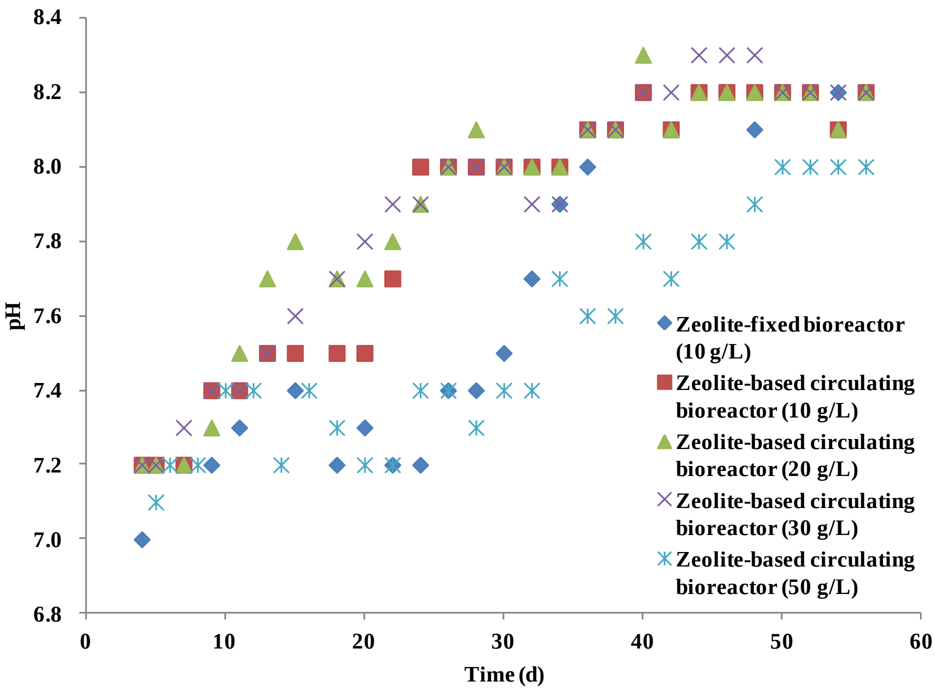


Figure 4. 5 The pH variation of the zeolite-based bioreactors and zeolite-fixed bioreactor as control for the anaerobic digestion of piggery wastes during the experiment.

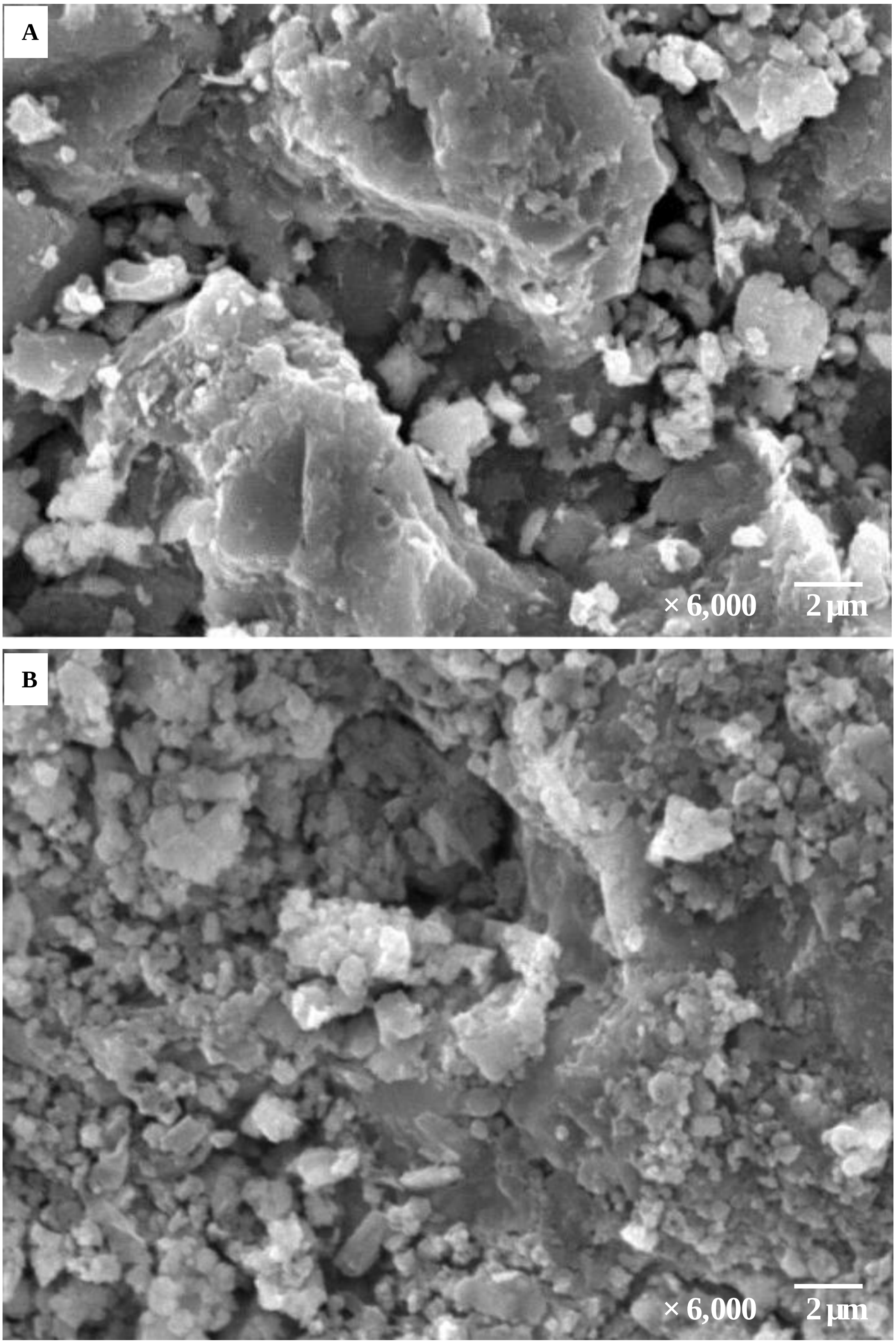


Figure 4. 6 SEM images of (A) artificial zeolite A-3 and (B) 30 g/L zeolite-based circulating bioreactor after anaerobic digestion.

Table 4. 1 Comparing the performance of the zeolite-fixed bioreactor and the zeolite-based circulating bioreactor.

	Start-up time (day)	Accumulated methane production (L)	Methane concentration (%)	Optimum zeolite dosage (g/L)
Zeolite-based circulating bioreactor	7th	2.68	59.4-89.0	30
Zeolite-fixed bioreactor	12th	2.30	61.3-93.3	10

Chapter 5 Conclusions

In the present study, the zeolite-fixed bioreactor and the zeolite-based circulating bioreactor were developed for methane fermentation of ammonium-rich piggery wastes. Firstly, the adsorption mechanism of ammonium on zeolite A-3 were carried out by kinetic and isotherm analyses. In addition, the desorption efficiency of ammonium from saturated zeolite A-3 was tested in sodium sulfate solution. Following, using zeolite-fixed bioreactor to mitigate ammonia inhibition for methane production from ammonium-rich piggery wastes was tested and explored the optimum zeolite dosage loading rate. Furthermore, the new zeolite-based circulating bioreactor was also investigation, whether could be efficient for reducing the lag period and enhancing methane production.

1 Adsorption and desorption studies on zeolite A–3

Zeolite is a common and typical adsorbent for ammonium removal. However, each special zeolite material has its special characteristics, thus investigate the detailed mechanisms of adsorption and efficiency of desorption on the synthesis zeolite A-3 is necessary. The following conclusions were obtained:

(1) Ammonium adsorption on zeolite A-3 fitted with the pseudo-second-order kinetic model (R^2=0.987) and can be described by both Langmuir (R^2=0.986) and Freundlich (R^2=0.985) isotherms. The maximum adsorption capacity of ammonium nitrogen on zeolite A-3 was 78.83 mg/g at an initial NH_4^+-N concentration of 5000

mg/L.

(2) The maximum desorption efficiency (38.2%) and highest effluent NH_4^+-N concentration (76.4 mg/L) were obtained under the equilibrium state. Desorption of ammonium from saturated zeolite fits the first-order (R^2=0.982) reversible reaction kinetic.

2 Improving anaerobic methane production from ammonium–rich piggery waste in a zeolite–fixed bioreactor and evaluation of ammonium adsorbed on zeolite A–3 as fertilizer

Ammonium adsorbent of zeolite also is a promising and potential carrier for immobilizing microorganisms, mitigating ammonia inhibition and enhancing methane yield. From batch experiment of methane fermentation in both zeolite-fixed bioreactor (dosage loading rate: 10 g/L and 30 g/L) and bioreactor without zeolite as control, the conclusions were draw as follows:

(1) The zeolite-fixed bioreactor demonstrated good performance, with methane yield of 354.2 mL/g-VS during all 33 days of the experiment at 35 °C and startup period on the 13th day.

(2) Using zeolite-fixed bioreactor could obviously decrease the startup period, enhanced methane yield and COD removal. In addition, the optimum zeolite loading rate 10 g/L was obtained.

(3) The bioreactor alleviated the ammonia inhibition during the methane fermentation of ammonium-rich piggery wastes via effective ammonium removal and

immobilization of microorganisms.

(4) Direct utilization of ammonium saturated zeolite as fertilizer could be increase the utilization efficiency of nitrogen fertilizer. Moreover, regeneration of zeolite A-3 using Na_2SO_4 solution also obtained a $(NH_4)_2SO_4$ by-product which is nice nitrogenous fertilizer.

3 Development of zeolite-based circulating bioreactor for anaerobic digestion of ammonium-rich piggery wastes

A new zeolite-based circulating bioreactor was developed for eliminating ammonia inhibition and enhancing methane production in the anaerobic digestion of ammonium-rich piggery wastes. In this part, it was investigated that whether the new zeolite-based circulating bioreactor could improve the anaerobic digestion efficiency and shorten the long lag phase.

(1) The zeolite-based circulating bioreactor could significantly shorten the startup period compared to zeolite-fixed bioreactor and enhanced methane production at dosage loading rates 20 g/L and 30 g/L.

(2) The methane production of the zeolite-based circulating bioreactors (zeolite dosage loading rate: 10 g/L, 20 g/L, 30 g/L and 50 g/L) were 5.15 L/L, 6.27 L/L, 6.69 L/L and 4.21 L/L for 56 days, respectively. According to methane production, the optimum zeolite loading rate of the zeolite-fixed bioreactor was 30 g/L in current study.

(3) Due to characteristic of the zeolite-based circulating bioreactor, zeolite was

more easily picked up as fertilizer directly or indirectly.

4 Further research

The present study developed two novel bioreactors as zeolite-fixed bioreactor and zeolite-based circulating bioreactor for methane fermentation of ammonium-rich piggery wastes. From the forethought of practical application, continuous test is suggested for future research. Besides that, the role of zeolite in the zeolite-based circulating bioreactor was only used as ammonium adsorbent. Fixed zeolite plays an important role on microorganism immobilization in the zeolite-fixed bioreactor. Therefore, in the future, zeolite-fixed circulating bioreactor should be developed for ammonium-rich methane fermentation by effective ammonium removal and microorganism immobilization.

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Acknowledgements

I wish to express my sincere appreciation to my principal advisor, Professor Zhenya Zhang, for his guidance, encouragement, and enthusiasm during my studies at Tsukuba for six years. His professionalism is an excellent I can follow throughout my career. I also wish to express my appreciation to my co-supervisor, Associate Professor Yingnan Yang, for her guidance, expertise and friendship during my studies. Her professionalism and hardworking is an excellent example I can also follow throughout my career.

I also would like to express my great appreciation to my dissertation committee members, Professor Zhenya Zhang, Associate Professor Motoo Utsumi, Associate Professor Helmut Yabar and Associate Professor Yingnan Yang for their numerous suggestion, comments, willingness and helpful discussion to serve as committee members. All their instructors have provided great insight and a memorable learning experience at University of Tsukuba that I will remember for the rest of my life.

Many friends and fellow students deserve recognition for their direct and indirect contributions to this thesis. I wish to express my appreciation to Qinghong Wang, Dawei Li, Yingxin Zhao, Hanying Zheng, Yuepeng Wang, Dan Zhu, and many others for their countless assistance during the years of my study here.

Finally, I reserve much of my appreciation for my parents and my wife Yuan Li. This dissertation would not have been completed without their love and support.

Appendix

1. **Cang Yu**, Dawei Li, Qinghong Wang, Zhenya Zhang, Yingnan Yang. Improving anaerobic methane production from ammonium-rich piggery waste in a zeolite-fixed bioreactor and evaluation of ammonium adsorbed on zeolite A-3 as fertilizer. International Journal of Waste Resources, Volume 4, Issue 4, Page 1-8. (doi:10.4172/2252-5211.1000160)

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